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STUDIES ON TOMATO WILT CAUSED BY FUSARIUM LYCOPERSICI SACC.[1]

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INTRODUCTION

That the tomato-wilt disease caused by *Fusarium lycopersici* Sacc. is becoming more important each year to the tomato-growing industry is evidenced by the reports which have appeared in the "Plant Disease Reporter" for the past several years. Not only is the disease of increasing importance in sections where it has already become established, but it is gradually spreading to new localities. It has been present in the Chautauqua region of New York since 1915,[3] was reported as being rather general in its distribution but causing only slight damage in 1921 (*18, p. 319*),[4] and as causing an estimated damage of 2.5 per cent in 1922 (*10, p. 56*). Minnesota reported the disease present for the first time in 1923, a damage of 10 to 20 per cent having been noted in one field (*58*). While primarily a disease of the warmer regions of the country, especially in the Mississippi and Ohio River valleys, it is of increasing economic importance in the cooler tomato-growing regions of New York, and probably of other northern tomato-growing States. Judging from the reports from Minnesota in 1923, the disease may reach serious proportions even in the cooler sections of the country, especially during seasons when high temperatures are prevalent.

There are at present several varieties of tomatoes, either selections of resistant genotypes from standard varieties, or new varieties acquired through hybridization, which are very resistant to the wilt disease. Notable among these are the Marvel, Norton, Norduke, Louisiana Red, and Louisiana Pink varieties. There are many other selections or hybrids now obtainable from the various experiment stations where tomato wilt has been under investigation, which appear to be more or less resistant. These resistant varieties have been distributed from State to State and, in the majority of cases, have retained their resistance under the conditions of the new environment. This, however, has not always been the case.

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[2] Thanks are due the plant pathologists who have kindly supplied the writer with cultures of Fusarium isolated from wilted tomato plants. The writer also expresses his gratitude to Bernice Johnson, formerly of this institution, who assisted materially in much of the routine work connected with the studies herein reported, and to L. M. Massey, of Cornell University, for critical reading of the manuscript.

[3] Letter from C. Chupp, assistant professor of plant pathology, Cornell University, dated May 27, 1924.

[4] Reference is made by number (italic) to "Literature cited," p. 236.

The writer has observed that some strains or selections of tomato varieties, resistant in other States, have not proved resistant in Kansas (*60*). A striking example was noted with two lines of Bonny Best, selected for resistance in Ohio, which proved extremely susceptible when grown in Kansas. The Tennessee Beauty, selected by Essary for resistance in Tennessee, proved to be resistant in Ohio (*29*), showed intermediate resistance in California (*50*), but proved to be very susceptible in Kansas. Other cases similar to these are on record.

The cause or causes of this apparent "loss" of resistance may be: (1) Environmental changes which affect either the host or the pathogene or both and introduce factors which modify the character of resistance, or (2) the presence of distinct physiological races of the pathogene in different localities.

REVIEW OF LITERATURE

McClintock (*35*) called attention to the fact already mentioned that strains of tomatoes resistant to the wilt disease in one State or locality are not always resistant in other States or localities.

Shapovalov and Lesley (*50*) recently reported that "Possibly then a few varieties, or perhaps more precisely selections, from other States fail to develop their wilt resistant qualities under California conditions." Clayton (*11*) likewise noted variations in the pathogenicity of strains of *Fusarium lycopersici* isolated from Ohio, Tennessee, Maryland, and Indiana. Edgerton (*15*) stated that different strains of the tomato-wilt fungus did not show the same virulence, or pathogenicity, when tested in the greenhouse.

Leach (*33*) was unable to distinguish between three strains of *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. by means of the relation between temperature and growth or hydrogen-ion concentration and growth, but he found a wide difference between one strain and the other two when growing on a liquid medium containing various sources of carbon.

Hursh (*30*) has demonstrated that urediniospore germination of two biologic forms of *Puccinia graminis tritici* differed considerably in their response to temperature and to hydrogen-ion concentrations. The form more restricted in its host range was also found to be more limited in tolerance of extremes of hydrogen-ion concentration and of temperature as determined by urediniospore germination. He concludes that "at least some biologic forms apparently possess individual physiologic characteristics demonstrable by physical and chemical studies, and these characteristics alone may be sufficient to establish them as definite taxonomic entities."

Matsumoto (*37*, *38*) found that different isolations of *Rhizoctonia solani* varied physiologically. LaRue and Bartlett (*31*), working with *Pestalozzia guepini*, demonstrated that by precise methods the species could be broken up into an indefinite number of strains, the number depending only upon the precision of their methods. Harter and Weimer (*25*), studying 18 strains of *Rhizopus nigricans* from different parts of the United States and Europe, all equally parasitic on sweet potatoes, were able to separate one strain from the others by means of a study of their temperature relations. One strain was found to have a lower optimum and maximum temperature for growth than the other 17.

SCOPE OF INVESTIGATION

It is not improbable that the different degrees of pathogenicity exhibited by various strains of an organism are correlated with some physiological difference or differences among them. Working on this assumption, the relations of temperature and of hydrogen-ion concentration to growth have been studied in 24 strains [5] of Fusarium isolated from wilted tomato plants received from various parts of the country. An attempt has been made to correlate the results of these studies with the different degrees of pathogenicity that the strains exhibited in the greenhouse tests. The characteristics of the strains on various media are recorded in this paper. Studies have been made of carbon sources, the method of utilization of the carbon source, and the production and isolation of toxic substances produced as a result of growth upon synthetic media.

SOURCE OF CULTURES

The cultures used in these experiments are listed below. The numbers are those under which the several isolations are entered in the collection of the department of botany and plant pathology of the Kansas State Agricultural College.

In the work reported here the various strains will be referred to by number only, unless it is deemed expedient to do otherwise.

Accession No.	Locality in which found	Date isolated	Accession No.	Locality in which found	Date isolated
60	Cherryvale, Kans.	June 19, 1922.	129	Missouri	Received in 1923.
61	Hutchinson, Kans.	June 23,1922.	130	Arkansas	Do.
68	Manhattan, Kans.	July 17, 1922.	131	New York	Do.
119	Ames, Iowa	Received in 1923.	132	Ohio	Do.
120	Texas	Do.	133	Manhattan, Kans.	Sept. 8, 1923.
121	Tennessee	Do.	134	St. John, Kans.	Aug. 17, 1923.
122	Louisiana No. 399	Do.	143	Tennessee	Received in 1923.
123	Virginia	Do.	144	Wisconsin	Do.
124	Louisiana No. 57	Do.	145	Ohio	Do.
125	Louisiana	Do.	149	Georgia	Isolated in 1917.
126	New Jersey	Do.	150		Subculture of Wollenweber's type.
127	Louisiana No. 397	Do.			
128	New Jersey	Do.			

PATHOGENICITY OF THE STRAINS

All the strains of Fusarium which were used in this work were pure lined by the usual methods. All were tested in the greenhouse to prove their pathogenicity. That some strains are more virulent and more destructive than others in their attack upon seedling tomatoes is clearly evidenced by the behavior of seedlings when grown in the greenhouse in sterilized and in artificially inoculated soil.

The soil used was sandy loam sterilized for 60 minutes in an autoclave at 15 pounds pressure. After sterilizing, it was inoculated by mixing with each 4-inch pot of soil approximately 50 gm. of of a 3-weeks-old wheat-kernel culture of the organism. Seed of the Bolgiano Red tomato was sown immediately in each pot. The plants

[5] By "strains" as used in this paper is meant merely separate isolations from different localities in the United States. The term does not imply physiological races, unless so stated.

were kept in a greenhouse in which the temperature was not constant, the fluctuation during these experiments being between 75 and 85° F.

At the end of four weeks symptoms of wilt became evident on plants growing in soil inoculated with cultures 122, 127, 131 and 144. At the end of six weeks these were badly wilted and No. 120 was beginning to show some wilting. At the end of eight weeks all the plants in each pot were counted, cut at the surface of the ground with a sharp scalpel, and examined for symptoms of fibrovascular browning. At this time the plants growing in soil inoculated with strains 122 and 127 were completely dead, and those in soil inoculated with strains 131 and 144 were practically dead. The plants in soil inoculated with strain 120 were very badly wilted and a few plants in this pot were also dead. The remainder of the plants showed only a slight wilting and a large number of yellowed leaves. Yellowed leaves, however, in this particular experiment can not be taken as a symptom of wilt, since control plants, growing in sterile soil, showed some yellowed leaves at the end of eight weeks caused by crowding in the pot. In Table 1 are given the percentages of seedlings showing fibrovascular browning at the end of eight weeks. The germination of the seed used was very uneven, and different numbers of plants were therefore obtained in the several pots.

TABLE 1.—*Percentage of seedlings of Bolgiano Red tomatoes showing fibrovascular browning after eight weeks growth in infested soil*

Strain No.	Total number of plants	Number showing browning	Per cent diseased	Strain No.	Total number of plants	Number showing browning	Per cent diseased
60	10	6	60	129	29	3	10
61	25	2	8	130	25	5	20
68	17	8	47	131	19	17	89
119	24	6	25	132	19	1	5
120	31	18	58	133	32	6	19
121	17	15	88	134	7	2	29
122	20	20	* 100	143	28	2	7
123	26	12	46	144	17	14	82
124	15	3	20	145	9	0	0
125	34	5	15	149	15	14	93
126	32	15	47	150	24	20	83
127	17	17	* 100	Control	55	0	0
128	26	17	65				

* Tops dead and dry.

It is evident from Table 1 that all the strains of this organism do not attack seedling tomato plants with the same virulence. Strains 120, 122, 127, 131, and 144 are outstanding in that they produced wilt before any of the other strains and the plants infected were more severely wilted than the plants infected with other strains. Strains 121, 149, and 150 were exceptional in that while the plants were badly infected, as evidenced by the high percentage of fibrovascular browning, they showed no wilting. Strain 145, a strain isolated in Ohio and received from C. D. Sherbakoff, failed to cause any fibrovascular browning or wilting. However, it was similar to pathogenic strains in all cultural habits, as far as these were studied. As a result of these pathogenicity tests, strains 120, 122, 127, 131, and 144 are placed together as a group and designated as the 120 group. All other strains are arbitrarily placed in a second group designated as the 60 group.

CULTURAL CHARACTERISTICS OF THE STRAINS

The strains placed in the 120-group differ from the strains in the 60-group in several ways. They produced only a slight pink color, on acidified potato-dextrose agar. All the other strains produced a deep-vinaceous color on this agar, typical of Fusaria in the Elegans section as described by Sherbakoff (*51*) (pl. 1). Furthermore, on potato agar strains in the 120 group produced an abundance of microconidia in 10 days, giving the culture a powdery appearance, while all the other strains produced no spores of any kind in that length of time on the same medium. Another constant difference between the 120 group and the 60 group was observed when the strains were grown on sterilized wheat kernels. The 120 group produced an abundance of macroconidia in sporodochia, giving to the kernels a faint pink to a deep vinaceous-red tinge. All the other strains, when grown on sterilized wheat kernels, produced few macroconidia but an abundance of microconidia. They also produced a purplish vinaceous color of varying intensity. On steamed rice, the 120 group produced a purplish vinaceous color but less intense than that produced by the 60 group.

TABLE 2.—*Notes on 14-months-old cultures of Fusarium lycopersici growing on potato-dextrose agar in test tubes 380 cm. long (notes taken June 29, 1925)*

[Plus sign present; minus sign absent]

Culture No.	Zonation [a]	Color [b]	Habit of growth of aerial mycelium	Sclerotia [c]	Macroconidia	Microconidia [d]	Chlamydospores [e]	Remarks
60	Very slight	None	Very slight	−	−	−	+	
61	do	do	do	−	−	+	+	
68	Slight	Faint	Absent	−	+	+	+	Macroconidia very scarce.
119	Very definite	Medium	do	−	−	+	+	
120	None	None	Rather abundant.	+	−	+	+	
121	Slight	Faint	Absent	−	−	+	+	
122	None	None	Medium	+	−	+	+	Sclerotia rare.
123	Very definite	Slight	do	−	−	+	+	
124	None	None	do	−	−	−	+	Chlamydospores very few.
125	Irregular	Medium	Slight	−	−	+	+	Microconidia scarce.
126	Slight	Faint	Absent	−	−	+	+	
127	None	None	Moderate	+	−	+	+	Microconidia very abundant.
128	Definite	Medium	Absent	−	−	+	+	
129	Very definite	Deep	do	−	−	+	+	
130	None	None	Moderate	−	+	+	+	Chlamydospores and macroconidia scarce.
131	do	do	Abundant	+	−	+	+	Sclerotia rare.
132	do	do	Very slight	−	−	−	−	
133	Slight	Faint	Absent	−	−	+	+	
134	None	None	Slight	−	−	+	+	
143	Slight	Faint	Absent	−	−	+	−	
144	None	None	Abundant	+	−	+	+	Microconidia very abundant.
145	do	Very faint	Absent	−	−	+	+	
149	Faint	Faint	Abundant	−	−	+	+	
150	Definite	Medium	Slight	−	−	+	+	Chlamydospores very abundant.

[a] Bands of color usually delimited sharply; No. 125 only roughly zonate.
[b] Color typical of Elegans section when present; varying degrees of vinaceous purple.
[c] Sclerotia small and colorless.
[d] Microconidia oval continuous or 1-septate.
[e] Chlamydospores smooth, thick walled, colorless to brown, terminal or intercalary, often in pairs or in chains.

The strains were grown on potato-dextrose agar in tubes 380 cm. long with a 20 mm. diameter, in the dark at room temperature. Macroscopic and microscopic examinations of the cultures were made at the end of 14 months, and the results of these are given in Table 2. Wide variations were noted between the different strains. For example, they varied from nonzonate to very definitely zonate, from colorless to deep vinaceous purple, and from prostrate vegetative growth to an abundant production of aerial mycelium. Strains in the 120 group were alike in the lack of zonation, lack of color production, and the presence of abundant aerial mycelium. These characters were not, however, confined to these five strains, and so they can not be distinguished from the remaining strains by macroscopic characters alone. The production of sclerotia, however, was confined to these five strains, no other strain ever producing them during the course of these investigations. The objection might be justly raised that these cultures, by the lack of macroconidial production, were not cultures approaching the norm. Furthermore, they may have varied from the norm in different degrees. Repeated systematic attempts have been made to secure these cultures in "Hochkultur" over a period of four years without satisfactory results.

At the higher temperatures in culture on potato-dextrose agar, the different strains varied more in growth habits than at temperatures approaching the optimum. This is shown by Plates 2 to 7, inclusive, which are photographs of the strains after six days' growth on potato-dextrose hard agar at 32°C. It should be noted here that strains 120, 122, 127, 131, and 144 appear white in the plate, but in reality they were very faintly tinged with pink. This pink tinge was always present in thalli of these strains on this agar at all temperatures, when grown in culture dishes. Strains 61 and 134, like the five strains mentioned above, showed only a slight color. The higher temperatures were conducive to the production of aerial mycelium, while the lower temperatures were conducive to the production of color. Strains 61 and 134, although evidently lacking the deep purplish vinaceous color typical of the Elegans section of Fusarium at 32° C., developed it at temperatures of 24° and below. However, in common with strains 60, 129, and 132, they never developed such a deep color as did the other strains included in this group.

Strains 144 and 150 were selected as representative of the two groups and were grown on four agars suggested by Sherbakoff (*51*) to give normal cultures. The observations made on these cultures are presented in Table 3. The fact that strain 150 was not observed in a good sporulating condition on any of these media and that strain 144 produced macroconidia in abundance only upon oatmeal agar, makes a comparison of the two strains somewhat unsatisfactory. The data are presented, however, in conjunction with other data upon the strains to indicate that variations may be expected when studying different isolations of organisms causing tomato wilt.

TABLE 3.—*Spore measurements of 6-weeks-old cultures of strains 144 and 150 grown on different agars*

Agar used	Measurements of strain 144 (microns)	Measurements of strain 150 (microns)
Rice	Microconidia: Maximum 17.0. Minimum 5.1. Average 9.55 (100).[a] Macroconidia, none. Chlamydospores: Maximum 14.0. Minimum 7.22. Average 9.77 (40).	Microconidia: Maximum 15.3. Minimum 6.8. Average 10.91 (50). Microconidia, none. Chlamydospores: Maximum 12.75. Minimum 6.80. Average 9.21 (40).
Lima bean	Microconidia: Maximum 17.0. Minimum 3.4. Average 8.87 (50). Chlamydospores: Maximum 13.6×8.5. Minimum 6.8×6.8. Average 9.82×8.02 (25). Macroconidia: Maximum 34.0. Minimum 11.9. Average 21.28 (25).	Microconidia: Maximum 17.0 Minimum 5.1. Average 9.69 (50). Chlamydospores: Maximum 11.9×8.5. Minimum 5.95×5.52. Average 8.17×7.07 (25). Macroconidia, none.
Wheat	Microconidia: Maximum 20.4. Minimum 3.4. Average 8.46 (50). Chlamydospores: Maximum 11.9. Minimum 6.8. Average 9.77 (25).	Microconidia: Maximum 15.3. Minimum 5.1. Average 8.87 (50). Chlamydospores: Maximum 10.2×5.1. Minimum 5.1×5.1. Average 7.70 (25).
Oatmeal	Microconidia: Maximum 13.6. Minimum 3.4. Average 7.31 (50). Chlamydospores: Maximum 11.9. Minimum 6.8. Average 9.19 (50). Macroconidia (sporodochia): Maximum 51.0. Minimum 18.7. Average 39.27 (200).	Microconidia: Maximum 13.6. Minimum 6.8. Average 9.31 (50). Chlamydospores: Maximum 10.62. Minimum 6.8. Average 8.50 (50). Macroconidia, none.

[a] Numbers in parentheses indicate number of spores measured from which the averages were obtained.

PHYSIOLOGICAL STUDIES ON FUSARIUM LYCOPERSICI

TEMPERATURE AS RELATED TO GROWTH

A great deal of work has been done upon the temperature relationships of pathogenic organisms and the diseases which they cause. No attempt will be made here to review the great mass of literature that has accumulated upon this subject, except where pertinent to this study.

The object of studying the temperature relationships of a number of strains of Fusarium which had been isolated from wilted tomato plants in various parts of the country was to determine if by such a study constant physiological differences between strains could be discovered. Brown (*8*), speaking of the problems that may be solved by a study of temperature relations, states that one of these is the problem of physiologic races. "Accepting the existence of several strains of the same organism, what physiological differences can one establish between them, and can one correlate with these any of the distinct appearances of the particular strains?"

Edgerton and Moreland (*17*), working with a Louisiana strain of *Fusarium lycopersici* on bean-pod agar, determined the optimum temperature for growth over a five-day period to be 29° C. Clayton (*11*),

working with an Indiana strain on potato hard agar, determined the optimum for growth to be 28°. It is evident that this organism is one requiring relatively high temperatures for maximum growth over a short period of time when grown on artificial substrata. That this relation may not hold for growth in soil over longer periods of time is possible since Balls (*3*) found that the optimum temperature for growth of a fungus is a variable factor, depending on the physiological history of the fungus as well as upon the immediate conditions under which it is growing. In this connection, however, Clayton (*11*) has demonstrated that the optimum soil temperature for the disease is approximately 28°, while at temperatures above 33° and below 21° it is practically inhibited. That the substrate upon which the organism is growing is one of the most important of the factors that influence growth is probable. Brooks and Cooley (*7*), studying the temperature relations of several apple-rotting fungi under different conditions, concluded that the temperature response of a particular organism was modified by the medium upon which it was grown. The minimum temperature for the various fungi studied varied greatly with the food material, but there was little variation in the optimum under the same conditions. Link (*34*) found in a study of Fusaria related to potato tuber rot that the optimum and maximum temperature points varied with the medium used. His results were corroborated by Goss (*24*), who stated as a result of his studies upon the temperature and humidity relations of some Fusaria rots of the Irish potato, that the cardinal points may vary with the medium used. It might be expected, therefore, that the strains of the same organism from widely different sections of the country, representing the northern and southern limits of its geographical range, would differ in their physiological behavior when brought together and subjected to the same conditions.

Fawcett (*19*) has summed up the conditions that influence the rate of growth of a fungus in culture and pointed out that if one of these is to be studied the others should be definitely stated. The conditions that should be given are the nature of the fungus, the nature of the medium, temperature, radiation, and duration.

Experimental Methods

The medium used for this work was hard potato-dextrose agar, made according to the formula of Hopkins (*27*).[6] The medium was prepared as needed.

The temperature desired was maintained in a low temperature incubator. Constant temperatures were used, and a fluctuation of not more than 0.5° from the temperature desired was recorded at any time. The cultures were kept in the dark at all times, being removed only for daily measurements. Fawcett (*19*) had previously shown that the removal of cultures for a brief period of time each day for measurements does not influence the growth rate. The duration factor was investigated before the final experiments were made. That this factor is of importance in measuring physiological processes has been recorded recently in a number of papers (*3*, *8*, *19*, *47*, *48*).

[6] Two hundred gm. of cubed potatoes brought to a boil in 1,000 c. c. of tap water. To the strained potato broth was added 10 gm. of dextrose and 20 gm. of agar.

The duration of the time interval chosen for this work was four days. Growth was allowed to take place for two days before experimental measurements were taken. It was found that with the temperatures and fungi used the time interval or the relation of this interval to the entire culture period had little significance. The same results would have been obtained if the measurements of all the days in the culture period had been averaged rather than those chosen, as will be brought out later in this paper.

From the stock cultures of the strains of the organism subcultures were made in Petri dishes. These were held for three days at the temperature which was to be used for the experimental cultures except at the lower temperatures, where five days were used. A small piece of agar with mycelium from just back of the growing margin of the colony was transferred from these subcultures to the experimental dishes, which were then inverted and placed in the culture chamber of the incubator. With temperatures below 28° C. no difficulty was experienced in keeping the humidity of the chamber at a point which prevented drying of the medium. With temperatures above 28° water was atomized into the chamber three times a day. A relative humidity of approximately 85 per cent was maintained in the culture chamber by observing these precautions. Humidities were measured by means of a wet-bulb apparatus. Gradual drying of the medium, it was found, would decrease the growth rate of the organism, and the colony would then have the appearance of a staled colony, as described by Brown (*8*). This decrease in growth rate may have been due to a deficiency of moisture in the medium or to a decrease in the diffusion of food materials through the more concentrated substrate.

In taking the measurements the diameter of the colony was measured at the same hour on consecutive days. The advantages of this method of measuring growth over the dry-weight method have been pointed out by Brown (*8*).

In getting the weekly growth rates, tubes 380 cm. long with a 20 mm. inside diameter were used. A piece of cork was cemented in the open end of the tubes about 60 mm. from the mouth. Hard potato-dextrose agar was poured in and the tubes plugged and sterilized. They were then placed in a horizontal position until the agar had solidified, resulting in an even layer of agar the entire length of the tube. The agar in the tubes was inoculated with the strains and placed in a horizontal position. The tubes were kept at room temperature in the dark, the maximum and minimum for each 24-hour period being recorded. Weekly increases in growth were also recorded.

Experimental Results

A summary of the results of the measurements of daily growth rates of the organisms studied appears in Table 4. Two diameters of each colony were measured at the end of each 24-hour period. The average of these two measurements was taken to represent the average diameter of the colony at the time of measurement. The average of all the cultures used in each temperature series was computed and these averages appear in Table 4.

TABLE 4.—*Mean average of the daily growth rates (in millimeters) of 24 strains of Fusarium lycopersici at temperatures ranging from 10° to 35° C.*

Strain No.	Mean average of daily growth rate (mm.) at temperatures of—						
	10° C.	12° C.	15° C.	16° C.	18° C.	20° C.	22° C.
60	[a]0.87 (3)	1.64 (2)	3.08 (3)	3.66 (4)	3.20 (2)	4.84 (5)	3.50 (2)
61	1.19 (3)	.80 (2)	3.13 (3)	3.80 (4)	4.31 (2)	5.57 (4)	6.00 (2)
68	.56 (3)	1.03 (2)	2.12 (3)	2.97 (4)	2.81 (2)	4.25 (5)	4.58 (2)
119	1.04 (3)	1.28 (2)	2.37 (3)	3.00 (2)	3.31 (2)	4.99 (5)	5.00 (2)
120	1.29 (3)	1.36 (2)	2.69 (3)	3.59 (5)	4.36 (2)	6.08 (10)	4.70 (4)
121	.95 (3)	1.57 (2)	2.93 (3)	3.81 (3)	3.53 (2)	5.15 (5)	4.41 (2)
122	.95 (3)	1.50 (2)	2.31 (3)	2.63 (4)	3.90 (2)	5.54 (5)	5.16 (2)
123	1.10 (3)	1.50 (2)	2.48 (3)	3.43 (4)	3.65 (2)	5.60 (5)	4.83 (2)
124	1.09 (2)	1.41 (2)	2.39 (3)	3.00 (3)	3.69 (2)	4.53 (5)	5.00 (2)
125	1.17 (3)	1.46 (2)	2.28 (3)	3.81 (4)	3.75 (2)	5.82 (5)	5.83 (2)
126	1.01 (3)	1.21 (2)	2.36 (3)	3.13 (4)	4.04 (2)	4.86 (5)	6.00 (2)
127	1.07 (3)	1.28 (2)	2.40 (3)	3.61 (4)	4.21 (2)	5.69 (5)	5.66 (2)
128	1.01 (3)	1.13 (2)	2.32 (3)	2.84 (4)	3.70 (2)	5.16 (5)	5.25 (2)
129	1.37 (3)	1.43 (2)	2.66 (3)	3.39 (4)	3.69 (2)	5.41 (5)	6.25 (2)
130	1.15 (3)	1.34 (2)	2.57 (3)	2.76 (4)	3.67 (2)	5.11 (5)	5.75 (2)
131	1.08 (3)	1.23 (2)	3.30 (3)	3.95 (4)	4.34 (2)	6.27 (5)	5.25 (2)
132	.67 (3)	1.25 (2)	2.29 (3)	2.87 (4)	2.84 (2)	3.99 (5)	6.50 (2)
133	.79 (3)	1.27 (2)	2.65 (3)	3.09 (4)	3.65 (2)	5.18 (5)	5.33 (2)
134	.72 (3)	1.48 (2)	2.27 (3)	3.63 (4)	3.73 (2)	5.17 (5)	4.16 (2)
143	1.33 (3)	.98 (2)	2.62 (3)	3.52 (4)	3.67 (2)	5.40 (5)	----------
144	.94 (3)	1.39 (2)	3.05 (3)	4.11 (4)	4.15 (2)	6.13 (5)	----------
145	1.08 (3)	1.28 (2)	2.60 (3)	3.13 (4)	2.96 (2)	5.05 (5)	----------
149	.68 (3)	1.30 (2)	1.95 (3)	3.07 (4)	----------	2.57 (2)	----------
150	1.11 (3)	.86 (2)	2.13 (3)	2.94 (4)	----------	2.67 (2)	----------

Strain No.	Mean average of daily growth rate (mm.) at tenperatures of—							
	24° C.	25° C.	26° C.	28° C.	30° C.	32° C.	34° C.	35° C.
60	6.38 (3)	6.79 (3)	6.78 (4)	7.25 (2)	6.75 (4)	3.08 (2)	0.68 (3)	[b]
61	8.94 (5)	9.10 (3)	7.19 (5)	9.01 (4)	5.78 (4)	3.16 (3)	.99 (3)	[c]
68	7.42 (5)	5.83 (3)	6.21 (5)	5.71 (4)	6.00 (4)	2.37 (4)	.94 (3)	[b]
119	7.28 (5)	7.62 (3)	6.86 (5)	6.83 (4)	6.48 (4)	1.66 (4)	.34 (3)	[b]
120	7.25 (3)	7.75 (3)	7.54 (7)	7.40 (8)	6.71 (8)	3.39 (5)	1.06 (6)	0.28 (3)
121	5.57 (3)	6.22 (3)	6.41 (5)	6.25 (2)	4.93 (4)	2.03 (4)	.56 (3)	[b]
122	7.12 (5)	7.67 (2)	7.92 (4)	6.84 (4)	5.72 (4)	2.36 (3)	.93 (3)	.14 (3)
123	5.20 (3)	5.68 (3)	5.87 (5)	5.80 (4)	5.21 (4)	3.97 (3)	1.06 (3)	[b]
124	7.52 (5)	8.10 (3)	6.91 (6)	6.57 (4)	6.29 (4)	4.25 (3)	.67 (3)	[b]
125	6.28 (3)	7.48 (3)	7.08 (5)	6.66 (4)	6.34 (4)	3.10 (4)	.69 (3)	[b]
126	6.90 (5)	7.37 (3)	7.36 (5)	6.40 (4)	5.74 (4)	3.38 (4)	1.25 (3)	[b]
127	7.00 (5)	7.83 (3)	7.67 (5)	7.34 (4)	6.42 (4)	3.56 (3)	.68 (3)	.14 (3)
128	6.06 (3)	8.60 (3)	6.49 (5)	7.02 (4)	6.39 (4)	2.91 (4)	.62 (3)	[b]
129	7.63 (3)	8.58 (3)	8.54 (5)	7.96 (4)	8.32 (4)	4.97 (4)	1.12 (3)	.57 (3)
130	7.11 (5)	7.23 (3)	7.35 (4)	6.40 (6)	6.34 (4)	3.08 (4)	.34 (3)	[b]
131	8.62 (2)	8.41 (3)	7.30 (5)	7.53 (4)	6.90 (4)	3.20 (3)	1.68 (3)	[b]
132	7.24 (5)	8.02 (3)	6.59 (5)	7.59 (5)	7.01 (4)	3.55 (4)	.37 (3)	[b]
133	6.88 (5)	6.98 (3)	6.20 (5)	6.40 (5)	5.24 (4)	3.60 (4)	1.00 (3)	[b]
134	7.37 (2)	6.31 (3)	7.67 (4)	6.80 (4)	5.89 (4)	1.53 (2)	.43 (3)	[b]
143	6.42 (3)	7.37 (3)	7.75 (5)	6.81 (3)	7.15 (2)	3.41 (3)	----------	[b]
144	6.88 (3)	8.04 (3)	7.04 (5)	6.65 (3)	5.53 (2)	3.35 (3)	----------	.28 (3)
145	6.44 (3)	6.23 (3)	6.29 (5)	4.66 (3)	4.56 (2)	2.37 (2)	----------	.14 (3)
149	5.93 (3)	7.45 (3)	6.19 (3)	----------	5.51 (2)	2.79 (2)	----------	.14 (3)
150	6.20 (3)	6.54 (3)	4.85 (3)	4.79 (2)	4.65 (2)	1.71 (4)	----------	.21 (3)

[a] Numbers in parentheses indicate number of colonies measured from which the averages were obtained.
[b] Perceptible but not measurable growth.
[c] No growth.

From the data presented in Table 4, the growth curves of the several strains were drawn. These curves appear as Figures 1 and 2. Table 5 presents the average increase in diameter of the two groups of strains over the four-day growth period previously described. In Table 6 the weekly growth rates of the several strains over a six-weeks' period are shown, including the mean weekly temperatures and the average growth rates of the several strains, collectively and individually. Table 7 presents the mean 24-hour diameter increases of a few of the

strains for five-degree temperature intervals from 10 to 30° C. The average of 5, 6, or 7 day growth periods are also given. Owing to slight variations in the size of the inoculum used, it was thought best in this case also to discard the first day's growth of all cultures. The object of presenting these data, analyzed as they are, is to show that with these organisms the length of observation period, within the limits of these experiments, is not of major importance in computing average daily growth rates. In this connection also, the mean weekly temperatures and growth rates of these strains over a six-weeks period, as presented in Table 6, are of interest.

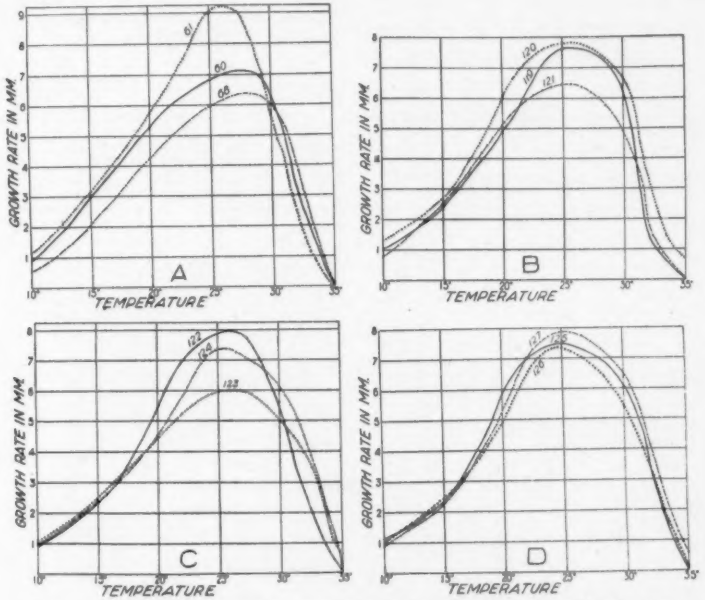


FIG. 1.—Temperature-growth curves (smoothed) of strains of *Fusarium lycopersici* Nos. 60, 61, 68, 119, 120, 121, 122, 123, 124, 125, 126, and 127

TABLE 5.—*Average daily increase in diameter (in millimeters), at different temperatures of the two groups of strains used in temperature studies*

Group	Average daily increase in diameter (mm.) at temperatures of—							
	10° C.	12° C.	15° C.	16° C.	18° C.	20° C.	22° C.	24° C.
120 group	1.06	1.35	2.75	3.59	4.19	5.94	*5.19	7.37
60 group	.99	1.27	2.48	3.26	3.54	4.81	6.61	6.77

	25° C.	26° C.	28° C.	30° C.	32° C.	34° C.	35° C.
120 group	7.94	7.49	7.15	6.25	3.17	1.09	0.32
60 group	7.18	6.75	6.60	6.03	2.99	0.73	.....

* Grown in a separate incubator in cool room.

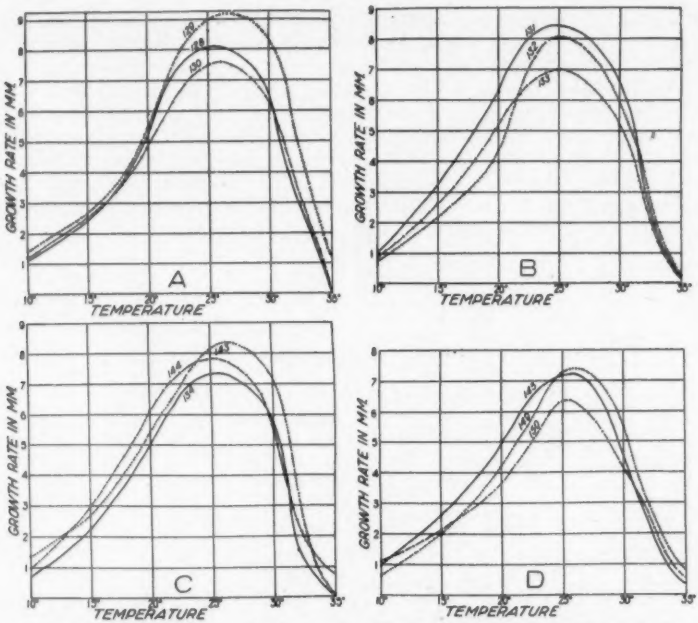


FIG. 2.—Temperature-growth curves (smoothed) of strains of *Fusarium lycopersici* Nos. 128, 129, 130, 131, 132, 133, 134, 143, 144, 145, 149, and 150

TABLE 6.—*Mean weekly temperatures and weekly growth rates of strains of Fusarium lycopersici*

Strain No.	Growth rate in millimeters						
	First week at 75.3° F. mean temperature	Second week at 72° F. mean temperature	Third week at 72.7° F. mean temperature	Fourth week at 76.25° F. mean temperature	Fifth week at 70.8° F. mean temperature	Sixth week at 74.6° F. mean temperature	Average
60	43	45	40	47	40	45	43.3
61	37	44	39	44	40	42	41.0
68	40	43	38	42	46	42	41.8
119	56	41	37	41	39	42	42.6
120	37	40	37	36	42	50	40.3
121	44	45	39	42	41	45	42.6
122	38	44	37	41	38	42	40.0
123	35	38	45	28	39	38	37.1
124	67	43	37	43	38	41	44.8
125	42	47	41	47	41	47	44.1
126	39	43	37	41	39	43	40.3
127	38	47	37	42	41	47	42.0
128	41	45	39	44	40	45	42.3
129	46	47	43	49	43	47	45.8
130	37	41	37	43	36	41	39.1
131	42	44	44	47	40	40	42.8
132	38	46	40	43	38	42	41.1
133	40	46	40	46	38	45	42.5
134	51	43	37	42	40	47	43.3
143	39	47	40	46	41	46	43.1
144	43	42	38	41	43	47	42.3
145	39	43	40	51	44	48	44.1
149	40	41	35	51	31	40	39.6
150	30	39	36	40	39	42	37.6
Average	41.7	43.5	38.8	43.2	39.8	43.9	----------

TABLE 7.—*Mean daily diameter increases in millimeters of mycelial disks of Fusarium strains isolated from wilted tomato plants*

Strain No.	24-hour period No.	Mean diameter increase at—				
		10° C.	15° C.	20° C.	25° C.	30° C.
		Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
60	1	1.15	4.08	5.75	7.50	7.06
	2	1.00	3.66	5.42	7.50	6.50
	3	1.17	3.00	5.42	6.33	6.75
	4	.58	2.79	5.08	7.00	5.62
	5	.71	2.79	4.92	6.33	6.12
	6	1.21	2.08	5.25		6.00
	7		2.42			
	Average entire period	.97	2.97	5.31	6.93	6.34
	4-day average	.86	3.06	5.21	6.79	6.25
119	1	1.29	2.58	5.08	7.83	5.75
	2	1.23	2.66	5.17	7.75	5.87
	3	1.21	2.33	4.58	6.92	6.50
	4	.87	2.29	6.25	7.83	6.87
	5	.87	2.21	6.08	8.00	7.62
	6	1.33	2.58	5.58		7.25
	7		2.00			
	Average entire period	1.13	2.38	5.45	7.67	6.64
	4-day average	1.04	2.37	5.52	7.62	6.71
120	1	1.58	3.16	7.34	8.83	7.12
	2	1.58	2.58	6.88	8.87	6.50
	3	1.54	2.92	6.79	7.54	6.37
	4	.88	2.71	7.71	7.25	6.25
	5	1.16	2.54	6.83	7.33	4.12
	6	1.16	2.87	7.00		5.87
	7		2.12			
	Average entire period	1.31	2.70	7.09	7.96	6.04
	4-day average	1.29	2.69	7.05	7.75	5.81
127	1	.92	3.16	6.00	9.00	3.75
	2	1.00	2.66	5.92	8.33	6.00
	3	1.50	2.33	6.33	7.33	7.25
	4	.58	2.21	5.66	8.33	5.12
	5	1.21	2.42	6.25	7.33	5.00
	6	1.04	2.23	6.33		5.37
	7		2.50			
	Average entire period	1.04	2.50	6.08	8.06	5.41
	4-day average	1.07	2.40	6.04	7.84	5.84
144	1	.88	3.87	7.21	9.33	5.69
	2	1.25	3.37	6.66	9.08	6.62
	3	1.00	3.17	6.66	7.46	6.93
	4	.87	2.83	6.75	8.37	3.94
	5	.63	2.84	6.42	7.25	4.62
	6	.92	2.92	6.58		5.50
	7		2.62			
	Average entire period	.92	3.09	6.71	8.29	5.55
	4-day average	.94	3.09	6.62	8.04	5.53
150	1	1.17	2.63	5.54	6.17	5.31
	2	1.17	2.46	5.00	5.42	4.37
	3	1.37	2.17	4.83	4.92	4.75
	4	1.00	2.33	6.17	5.75	4.50
	5	.92	2.38	5.42	6.08	5.00
	6	1.37	2.16	5.58		3.75
	7		2.13			
	Average entire period	1.16	2.32	5.42	5.67	4.61
	4-day average	1.09	2.33	5.35	5.54	4.65

DISCUSSION OF RESULTS

From the data presented in Table 4 and Figures 1 and 2 it is evident that the temperature for optimum growth over the four-day period under observation varies with the strain, the optima being within the limits of 24 to 28° C. For strain 60 from the southeastern part of Kansas the optimum is 28°, while for strain 131 from western New York the optimum is 24°. The other strains are grouped around 25 and 26° as their optimum growth temperatures.

As stated above, strains in the 120 group were distinctly more virulent in their attack upon seedling tomatoes in the greenhouse than the remaining strains. Certain other characters also differentiated

them from the strains in the other group. By reference to Table 5 it will be noted that at all temperatures strains in the 120 group were more rapid growers than those in the 60 group. Although both groups found their optimum temperature for growth at 25° C., the 120 group had a wider range of growth temperatures than the 60 group. This fact may explain why the strains of this group are found in New York and Wisconsin, as well as in Texas and Louisiana. From the latter State were obtained cultures of the organism which fell in both groups. One culture only was received from New York, one from Wisconsin, and one from Texas, and all fell in the 120 group.

The difference in the growth rates is widest at the optimum point, 25° C., a fact which is brought out in Figure 3. This difference in growth rate can be correlated with other characters; for example, lack of the production of a deep vinaceous red on agar rich in dextrose and the abundant production of macroconidia in sporodochia with a faint pink tinge when grown on wheat kernels.

The temperature curves of the various strains show wide differences. The most outstanding difference is noted in strain 129, isolated from a wilted tomato plant in Missouri by I. T. Scott. This organism grew with distinctly greater rapidity at the higher temperatures than the other strains.

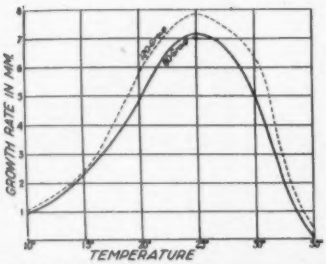


FIG. 3.—Temperature-growth curves of the two groups of strains of *Fusarium lycopersici* used in the temperature studies

Table 6 presents the mean weekly temperatures and the weekly growth rates of the 24 strains used in these studies. As can be readily seen, these fungi, when grown on potato-dextrose agar, are not of the staling type described by Brown (*8*). No evidence of a retarding influence on growth was noticed at any time during the six weeks in which these cultures were under observation. Fawcett (*20, 21*) found the same to be true of three fungi with which he worked. Being of the nonstaling type when grown on this medium, they should show, theoretically, no diminution in growth rate at the higher temperatures from day to day. That this was actually the case is shown by the data presented in Table 7. From this table it can be seen that the growth rates at the lower temperatures were very uniform from day to day, but that at the higher temperatures fluctuations took place. These can not be explained at this time. However, while in some cases there is an apparent decrease in growth rate with increase in duration at 30° C., in as many other cases there is an apparent increase. That the duration of the time interval used for obtaining the mean daily growth rates is of little importance in this case is evident from the averages presented in Table 7. With the four-day average taken after the initial two-day growth period is presented the average of the entire culture period of five to seven days. There is little difference between the two averages. With these fungi, therefore, it seems immaterial when determining the daily increases of growth by linear measurement whether a 4, 5, 6, or 7 day period is chosen from which to compute the daily mean.

HYDROGEN-ION CONCENTRATION AS RELATED TO GROWTH

The relation of acidity and alkalinity to growth of microorganisms has been studied in detail for a large number of species. For such a study on *Fusarium lycopersici*, and on the relation of the hydrogen-ion concentration of the soil to the development of the disease, we are indebted to Scott (*49*) and Sherwood (*52*).

Scott found that maximum growth of the organism was obtained at a P_H value of 4.5 to 5.3, followed by a minimum at P_H 5.25 to 5.8, with a second maximum at P_H 5.85 to 6.85. He also found in soil experiments that a minimum of wilt occurred at a P_H of 6.4 to 7.0, with a maximum of wilt on either side. This double maximum for growth and infection has also been found by Hopkins (*28*) for *Gibberella saubinetii*. Webb (*59*) has also found double maxima in his study of spore germination as influenced by the hydrogen-ion concentration.

Sherwood (*52*) determined the range of acidity and alkalinity tolerated by his strain of *Fusarium lycopersici* in tests upon spore germination to be P_H 2.2 to 8.4, which is wider than the range of hydrogen-ion concentration found in any ordinary soil. He did not find a minimum, however, in the amount of wilt produced, with a maximum of wilt on either side in his soil experiments. He did not present data upon the dry weights of mycelium produced at different hydrogen-ion concentrations, although it is evident from his notes that there was a minimum of growth at P_H 7.6 to 8.2 in one experiment and at P_H 6.6 to 7.0 in another, with a maximum on either side. These minimum points do not agree with that found by Scott (*49*), P_H 4.5 to 5.3.

In the studies reported here, not only were the hydrogen-ion ranges of the 24 strains of Fusarium isolated from wilted tomato plants determined, but dry weights of mycelium produced at different hydrogen-ion concentrations were also obtained.

EXPERIMENTAL METHODS

CULTURE SOLUTION

A modified Richards' solution was first used in the preliminary work. This was soon discarded, however, on account of the precipitation of magnesium phosphates in the alkaline end of the series. Tarr and Noble (*56*) called attention to this precipitation of phosphates above P_H 6, and their analyses of their solutions show that the precipitation was heaviest at P_H 8.

Uschinsky's solution was next tried, but the same difficulty as with Richards' solution was found. Furthermore, the growth of the several strains tried in this solution was very poor. Apparently the carbon sources supplied—ammonium lactate, sodium asparaginate, and glycerine—were relatively unavailable for these fungi.

A solution was finally synthesized upon which the organisms made a luxuriant growth, and in which there was no magnesium. The solution consisted of the following ingredients:[7] Distilled water 1,000 c. c.; NaCl 5 gm.; H_3PO_4 24.65 c. c. normal solution; KNO_3 10 gm.; potassium acid thalate 1.0207 gm.; dextrose 50 gm.

[7] In part after Tarr and Noble (*56*).

It was found that to prevent caramelization of the dextrose in the acid and alkaline solutions, it was necessary that the salt solution and the sugar solutions be made up separately, sterilized separately, and mixed when cool, under aseptic conditions. Consequently, the salt solution was made so concentrated that 40 c. c. of the new solution contained the same quantities of all the salts as 50 c. c. of the original.[8] A concentrated dextrose solution was also prepared so that 10 c. c. of the new solution contained as much dextrose as 50 c. c. of the original.[9]

PREPARATION OF CULTURE FLASKS

One hundred and twenty-five c. c. Erlenmeyer flasks were used. Forty c. c. of the salt solution was placed in each flask. The flasks were plugged and sterilized at 15 pounds pressure for 20 minutes. When cool, 10 c. c. of the dextrose solution, previously sterilized and cooled was poured in aseptically. The flasks were then incubated for 24 hours to detect contaminations.

ADJUSTING AND DETERMINING HYDROGEN-ION CONCENTRATIONS

The culture solution as prepared had a P_H value of 2.2 to 2.8. Adjustments were made by the addition of normal NaOH or normal KOH, (fig. 4). Stevens (*55*) has shown that these bases in common with NH_4OH have a low toxicity to fungous spores.

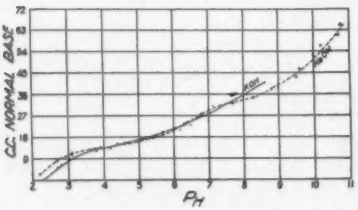


FIG. 4.—Titration curves of a modified Richards' solution with KOH and NaOH

A sample of the adjusted culture medium was sterilized with the culture flasks, and the dextrose solution was added to it in the same proportions as to the culture flasks. The hydrogen-ion concentration of this sample was determined in most cases electrometrically with the aid of the hydrogen electrode and potentiometer. In other cases the colorimetric method for the determination of hydrogen-ion concentration was used with equally satisfactory results. The color standards suggested by Medalia (*39*) were employed successfully in this connection.

INOCULATION OF CULTURE FLASKS

The flasks were inoculated by means of three loopfuls of a spore suspension containing some mycelial fragments. Wheat kernels upon which the organism had been growing for 10 to 14 days were shaken with 50 c. c. of sterile distilled water. This gave a heavy suspension of microconidia, except from the five strains in the 120-group; these gave a heavy suspension of macroconidia. The flasks after inoculation were placed in diffuse light at ordinary room temperature. Growth was allowed to proceed for 10 days, after which the cultures were filtered into previously prepared, dried, and weighed Gooch crucibles. The crucibles were then dried a second time at 100 to 105° C. for 24 hours and reweighed. The difference in weights before and after filtering was assumed to be a measure of the dry weight of the mycelium

[8] Formula for the concentrated salt solution: 1,000 c. c. distilled water; 6.25 gm. NaCl; 30.75 c. c. normal H_3PO_4 solution; 12.5 gm. KNO_3; and 1.2759 gm. potassium acid thalate.

[9] The concentrated dextrose solution contained 250 gm. of dextrose to 1,000 c. c. of distilled water.

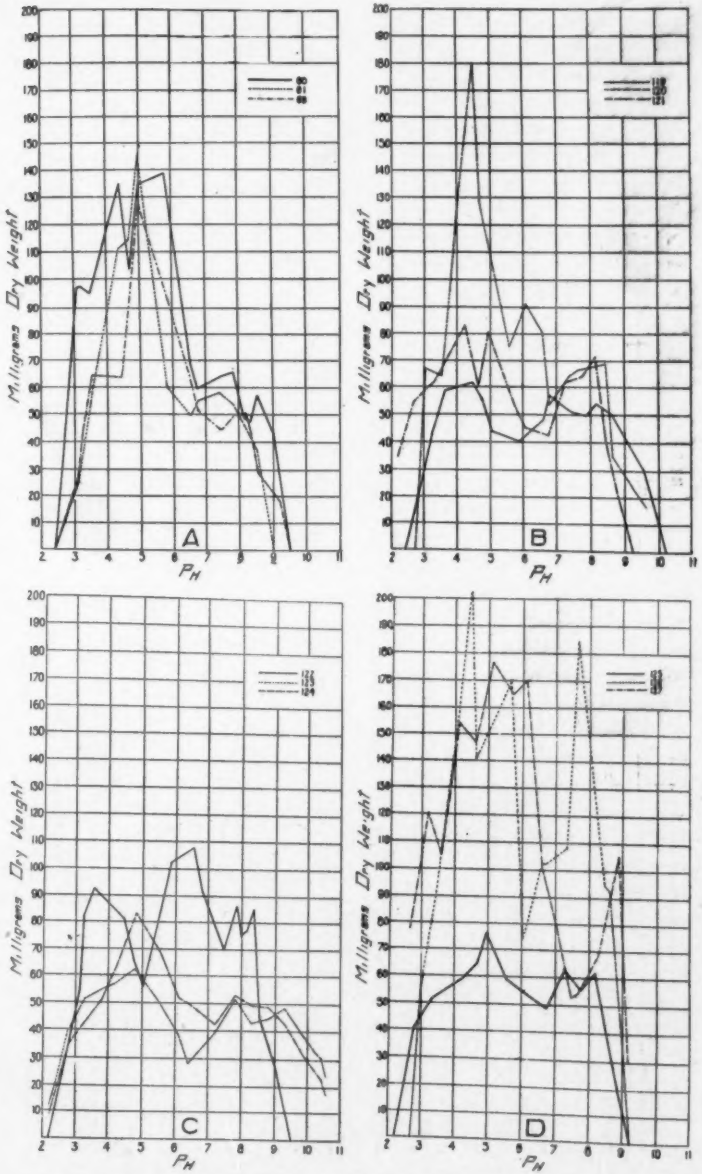


FIG. 5.—Hydrogen-ion-growth curves of *Fusarium lycopersici* strains Nos. 60, 61, 68, 119, 120, 121, 122, 123, 124, 125, 126, and 127

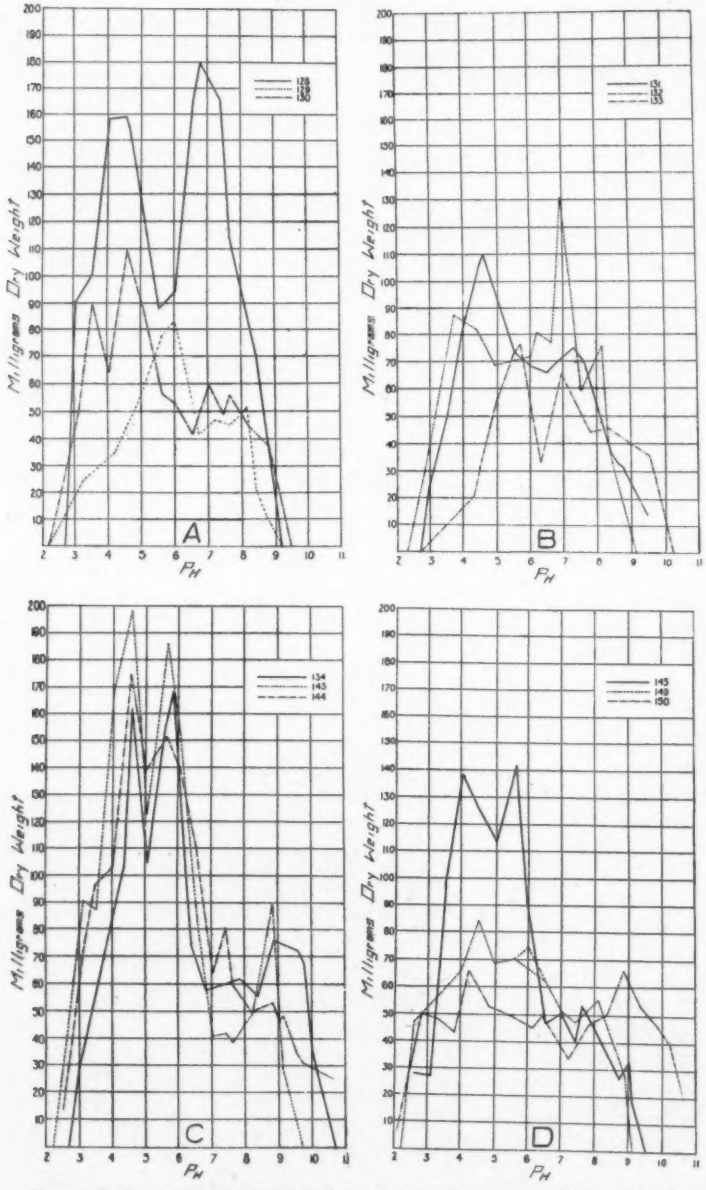


FIG. 6.—Hydrogen-ion-growth curves of *Fusarium lycopersici* strains Nos. 128, 129, 130, 131, 132, 133, 134, 143, 144, 145, 149, and 150

produced. The series were always run in duplicate, but only the average dry weight of the mycelium produced by the two cultures at any one hydrogen-ion concentration are recorded. It was from these figures that the hydrogen-ion-growth curves were plotted.

Experimental Results

The experimental data obtained appear in Table 8 and in Figures 5 and 6. In Figure 7, A are presented graphically the minimum and maximum hydrogen-ion concentrations tolerated by the strains where determined, and in Figure 7, B are shown the curves obtained for one strain when the tests were repeated three times.

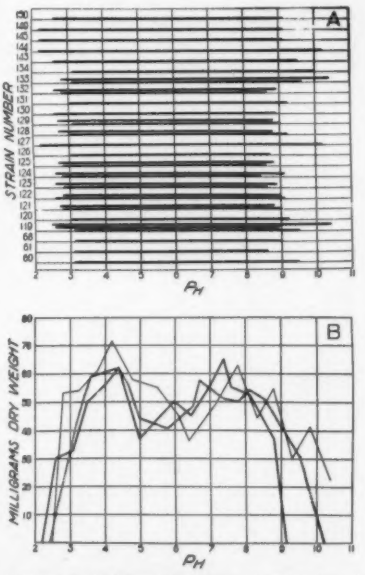


FIG. 7.—A, hydrogen-ion ranges of 24 strains of *Fusarium lycopersici;* B, hydrogen-ion-growth curves of strain 119, obtained from three repetitions at widely different times—December, 1923, March, 1924, and May, 1924

Discussion of Results

In analyzing these results the expected variation in dry weight due to the methods employed should be kept in mind. Brown (*8*) has pointed out the disadvantages of using dry-weight determinations as measurements of fungous growth, since under apparently identical conditions wide variations will frequently occur in the amount of dry weight produced. These irregularities were found to be more common and of greater extent as the limits of alkalinity were approached, and less so toward the limits of acidity. In this work, which involved such a large number of experimental cultures, the hydrogen-ion concentration of each was not determined. The cultures were prepared as described above in groups of nine, eight of which were used for inoculation, the ninth being reserved for the determination of the hydrogen-ion concentration. It was realized from the start that this method would involve slight errors. However, the hydrogen-ion concentrations of the groups were usually far enough apart so that no overlapping of individual cultures within two neighboring groups would result. The electrometric method of determining hydrogen-ion concentration was replaced by the colorimetric method during the latter part of these investigations. In addition to being more rapid than the electrometric method, it was accurate to within 0.1 P_H.

It should be borne in mind that these studies were conducted with several strains of the same organism, and the general appearance of the hydrogen-ion growth curves would be expected to be similar. Slight variations of the curve might be due to one of two things; either to physiological differences of the strains or to the possibility of errors arising, as just pointed out.

TABLE 8.—*Growth, expressed as dry weight in milligrams, of 24 strains of Fusarium lycopersici grown on a liquid medium for 10 days, at different hydrogen-ion concentrations*

Strain 60:																			
P_H values	2.4	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0	9.5	10.2	------	------
Dry weight	(a)	96.4	96.0	94.2	135.0	103.8	134.8	138.2	69.4	59.8	64.2	61.6	48.9	47.6	57.3	43.0	(b)	(a)	------	------
Strain 61:																				
P_H values	2.4	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0	------	------	------	------
Dry weight	(a)	25.5	32.6	57.7	112.3	114.7	146.5	60.2	50.0	55.4	57.6	53.6	41.5	38.0	37.5	(a)	------	------	------	------
Strain 68:																				
P_H values	2.4	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0	9.5	------	------	------
Dry weight	(a)	27.0	42.3	64.7	64.3	79.1	128.7	94.8	66.4	51.7	45.0	48.8	50.6	44.7	29.0	17.8	(a)	------	------	------
Strain 119:																				
P_H values	2.4	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	8.1	8.3	8.5	9.0	9.5	10.2	------	------
Dry weight	(a)	(b)	42.5	58.5	61.1	55.1	44.3	40.4	47.7	57.0	51.3	50.0	54.3	65.8	47.8	41.0	30.7	(a)	------	------
Strain 120:																				
P_H values	2.7	3.0	3.5	4.0	4.4	4.6	5.5	6.0	6.6	6.7	7.3	7.6	8.4	8.5	8.7	9.2	------	------	------	------
Dry Weight	(a)	67.0	64.4	133.6	180.9	128.1	75.3	91.7	81.0	53.9	64.7	66.7	68.7	35.4	33.3	24.3	------	------	------	------
Strain 121:																				
P_H values	2.2	2.7	3.3	4.2	4.6	5.7	6.0	6.7	7.7	8.1	8.4	8.8	9.2	------	------	------	------	------	------	------
Dry weight	34.7	54.7	62.0	83.5	60.5	53.4	45.8	42.7	64.2	71.3	41.3	20.0	(a)	------	------	------	------	------	------	------
Strain 122:																				
P_H values	2.2	2.8	3.1	3.2	3.5	4.4	4.7	5.0	5.8	6.5	6.7	7.4	7.8	7.9	8.1	8.3	9.5	------	------	------
Dry weight	(a)	(b)	56.5	82.9	92.8	81.9	66.5	56.4	103.0	108.1	92.8	71.2	87.1	76.5	78.2	85.9	(a)	------	------	------
Strain 123:																				
P_H values	2.5	2.8	3.3	3.8	4.2	4.8	5.5	6.1	6.4	7.2	7.8	8.3	8.8	9.3	9.8	10.2	10.4	------	------	------
Dry weight	(b)	39.9	52.1	45.1	57.5	63.0	51.2	39.4	28.0	40.0	51.6	42.9	44.7	48.7	39.7	32.7	30.8	------	------	------
Strain 124:																				
P_H values	2.5	2.8	3.3	3.8	4.2	4.8	5.5	6.1	6.4	7.2	7.8	8.3	8.8	9.3	9.8	10.2	10.4	------	------	------
Dry weight	(b)	35.0	43.0	51.3	62.4	83.8	69.5	52.1	50.0	42.8	53.5	49.6	49.2	42.8	32.3	33.3	22.8	------	------	------
Strain 125:																				
P_H values	2.2	2.7	3.3	4.2	4.6	4.9	5.7	6.0	6.7	7.2	7.7	8.1	8.4	8.8	9.2	------	------	------	------	------
Dry weight	(b)	39.9	51.7	59.3	65.0	76.5	59.8	54.5	49.0	63.6	56.8	62.3	44.2	20.5	(a)	------	------	------	------	------
Strain 126:																				
P_H values	2.7	3.0	3.5	4.0	4.4	4.6	5.5	6.0	6.5	6.6	6.7	7.3	7.6	8.4	8.5	8.7	9.2	------	------	------
Dry weight	(b)	56.1	97.3	142.1	203.3	140.8	171.9	74.0	99.2	104.0	103.0	108.7	185.6	95.9	92.9	(b)	(a)	------	------	------
Strain 127:																				
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.7	8.9	9.1	9.5	9.7	10.2
Dry weight	(a)	77.6	121.1	105.4	154.5	147.1	177.2	165.8	170.8	102.2	78.4	53.1	54.9	68.1	106.1	55.2	30.4	(b)	(b)	(b)
Strain 128:																				
P_H values	2.7	3.0	3.5	4.0	4.5	4.6	5.5	6.0	6.5	6.7	7.3	7.6	8.4	8.7	9.2	------	------	------	------	------
Dry weight	(a)	90.4	100.5	157.7	158.1	155.6	87.6	94.2	165.0	179.9	165.1	114.4	70.2	39.8	(a)	------	------	------	------	------
Strain 129:																				
P_H values	2.2	2.7	3.3	4.2	4.6	4.9	5.7	6.0	6.7	7.2	7.7	8.1	8.4	8.8	9.2	------	------	------	------	------
Dry weight	(a)	14.6	24.5	35.4	45.3	51.3	78.8	82.4	41.4	47.9	46.6	50.2	20.2	11.5	(a)	------	------	------	------	------
Strain 130:																				
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	------	------	------
Dry weight	(a)	(b)	51.1	89.6	63.9	109.3	89.2	56.3	53.5	42.4	59.7	48.9	56.8	45.6	37.9	(b)	(a)	------	------	------

Strain 131:																				
P_H values	2.7	3.0	3.5	4.0	4.4	4.6	5.5	6.0	6.6	6.7	7.3	7.6	8.4	8.5	8.7	9.2				
Dry weight	(a)	24.8	49.4	83.4	105.7	109.6	73.6	68.4	66.0	71.1	75.1	70.1	36.8	33.2	31.9	20.9				
Strain 132:																				
P_H values	2.8	3.7	4.4	4.9	6.0	6.2	6.6	6.9	7.5	8.1	8.3	8.4	8.6	9.1						
Dry weight	29.2	87.6	82.3	68.2	71.9	80.2	76.4	130.3	58.6	75.9	40.3	32.8	8.9	(a)						
Strain 133:																				
P_H values	2.7	3.1	4.2	4.4	5.0	5.4	5.7	6.3	6.9	7.8	8.3	9.5	9.7	10.0	10.2					
Dry weight	(a)	(b)	21.0	31.9	56.3	72.5	76.7	32.7	65.4	44.4	46.2	36.2	27.2	(b)	(a)					
Strain 134:																				
P_H values	2.7	3.1	4.2	4.4	5.0	5.4	5.7	6.3	6.8	7.7	8.3	8.8	9.5	9.6	10.0	10.2				
Dry weight	(a)	32.3	103.3	161.8	104.9	155.8	169.3	75.9	59.4	62.8	56.4	77.1	72.8	67.8	36.8	(a)				
Strain 143:																				
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	9.5	10.2	
Dry weight	(a)	(b)	91.9	87.6	168.9	199.4	122.8	187.3	152.3	86.2	41.3	42.2	38.6	49.8	90.0	54.6	29.7	(b)	(a)	
Strain 144:																				
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	9.5	9.7	10.2
Dry weight	(b)	20.1	68.3	96.7	103.5	173.0	139.7	152.0	140.0	111.0	64.5	81.1	60.8	49.1	53.1	46.9	48.4	35.1	31.4	27.7
Strain 145:																				
P_H values	2.2	2.6	3.1	3.5	4.0	4.4	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	9.5		
Dry weight	(b)	27.9	27.0	97.9	138.1	125.7	113.9	141.1	92.8	47.3	50.7	40.9	53.8	45.5	27.4	33.2	18.3	(a)		
Strain 149:																				
P_H values	2.2	2.6	4.0	4.5	5.0	5.6	6.0	6.5	7.0	7.4	7.6	8.1	8.8	8.9	9.1	9.5				
Dry weight	(b)	47.6	66.5	83.7	69.0	70.9	75.4	62.7	51.8	47.3	37.6	58.3	37.7	27.8	(b)	(a)				
Strain 150:																				
P_H values	2.5	2.8	3.3	3.8	4.2	4.8	5.5	6.1	6.4	7.2	7.8	8.3	8.8	9.3	9.8	10.2	10.4			
Dry weight	(b)	50.1	48.6	43.1	65.6	52.7	49.3	45.5	49.7	33.6	46.6	50.5	66.6	53.1	46.4	40.3	29.9			

[a] No growth.

[b] Slight growth.

All the strains showed a double maximum. The first maximum, while varying with the strains to some extent, appeared at P_H 4.0 to 5.5, and was followed by a minimum at P_H 5 to 7, with the second maximum at a point above P_H 7. This second maximum was found to be less regular in its position upon the P_H scale than the first. For example, the second maximum of strain 122 fell at P_H 6.6, while the second maximum of strains 134 and 143 fell at P_H 8.8. The second maximum of strain 144 as well as the minimum point was poorly defined, if present at all. Strains 122, 128, 132, and 150 are outstanding in respect to their second maximum. In these cases the second maximum is higher than the first, and in the first three cases mentioned, it fell just below neutrality. In strain 150, however, the second maximum fell at P_H 8.8, a point decidedly above neutrality.

It should be pointed out here that strain 150 in common with several others—119, 121, 122, 123, 124, 125, 129, 133, and 149—did not make the rapid and heavy growth in 10 days made by the remaining 14 strains.

Figure 7, B brings out an interesting fact observed during the course of these investigations. The maximum acidity tolerated and the first maximum growth point in the curve were found to be fairly constant, always appearing at nearly the same points on the P_H scale. However, the minimum growth point of the curve, the second maximum growth point, and also the maximum alkalinity tolerated were variable, and often varied between wide limits, especially the alkaline limit for germination and growth. The curves of Figure 7, B show these irregularities as well as the similarities of the curves obtained by three repetitions of this one strain. The irregularity of the maximum alkalinity tolerated is also expressed in graphic form in Figure 7, A.

Variations were also found in the growth ranges of the strains. For example, strain 61 has a short range (P_H 3.1 to 8.6) while others, especially strains 127 and 144, have long ranges (less than P_H 2.2 to more than P_H 10.2).

The writer has been unable to correlate the hydrogen-ion-growth curves with the temperature-growth curves or with the degrees of pathogenicity expressed by the several strains as presented in an earlier part of this paper.

SOURCES OF ENERGY FOR GROWTH

Different Sources of Energy

Two strains, namely, 144 and 150, were selected as representing the two arbitrary groups set up previously in this paper on a basis of virulence and macroscopic cultural characters, and were grown on Richards' solution with varying carbon sources at 2 per cent concentrations. The methods employed were similar to those previously used for measuring growth by dry-weight determinations.

The cultures were run in series of six. The figures which appear in Table 9 are the average of the dry weights obtained from the six cultures. Growth was allowed to continue for 10 days from the time of inoculation, after which the cultures were filtered into previously prepared and weighed Gooch crucibles, and dry-weight determinations were computed.

TABLE 9.—*Growth of strains 144 and 150 with different carbon sources*

Carbon source	Dry weight		Carbon source	Dry weight	
	Strain 144	Strain 150		Strain 144	Strain 150
	Mgm.	*Mgm.*		*Mgm.*	*Mgm.*
Arabinose	35.1	45.3	Galactose	44.5	38.5
Dextrose, 1 per cent	45.2	41.2	Levulose	70.1	64.7
Dextrose, 2 per cent	56.3	48.0	Lactose	18.7	15.9
Maltose	61.7	53.1	Dextrine (white)	66.2	58.1
Mannose	46.3	42.4	Dextrine (yellow)	75.6	85.5
Glucose	92.3	63.1			

In Table 9 it is shown that strain 144, which had made more rapid growth in previous experiments, again showed greater rapidity of growth. Strain 150 produced the greater dry weight over the time allotted, on arabinose and yellow dextrine. Although the difference between the two strains is slight, yet it is constant. Lactose served as a very poor carbon source for both strains.

In this connection should be noted the very poor growth made by several of the strains on Uschinsky's solution with ammonium lactate, sodium asparaginate, and glycerine as the only available carbon sources.

UTILIZATION OF DEXTROSE AS RELATED TO HYDROGEN-ION CONCENTRATION

It was the purpose of this series of experiments to follow the use of dextrose by *Fusarium lycopersici* during the growth period when different initial hydrogen-ion concentrations were used.

EXPERIMENTAL METHODS

Growth was measured by dry-weight determinations as described previously. Hydrogen-ion concentrations were determined electrometrically. Dextrose determinations were made by the general gravimetric method as recommended by the Association of Official Agricultural Chemists (*1*). The culture solution used was Richards' solution.[10] This solution as prepared had an initial P_H reading of approximately 4 after sterilization. Small quantities of dextrose were destroyed by the sterilization process (15 pounds pressure for 20 minutes), so that the initial concentration was approximately 4.5 per cent in all the series.

Series 1 had an initial P_H reading of 4.04. In series 2 the KH_2PO_4 was replaced by an equal amount of K_3PO_4. The initial P_H of the solution in this series was 7.15. In series 3, K_3PO_4 was used and the resulting solution adjusted by the addition of $Ca(OH)_2$ to an initial P_H of 9.04.

The flasks were inoculated with a spore suspension of *Fusarium lycopersici*, strain 144, and kept in diffuse light at laboratory temperature. During the period of most rapid growth dry weight determinations, sugar determinations, and hydrogen-ion determinations were made every other day in triplicate. As changes between determinations became less marked determinations were made less frequently.

[10] KNO_3, 10 gms.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 20 mgm.; dextrose, 50 gm.; distilled water, 1,000 c. c.

Experimental Results

The data obtained on the relation between growth, hydrogen-ion concentration, and changes in hydrogen-ion concentration caused by growth, and dextrose utilization are presented in Table 10. The data are also expressed graphically in Figures 8 and 9.

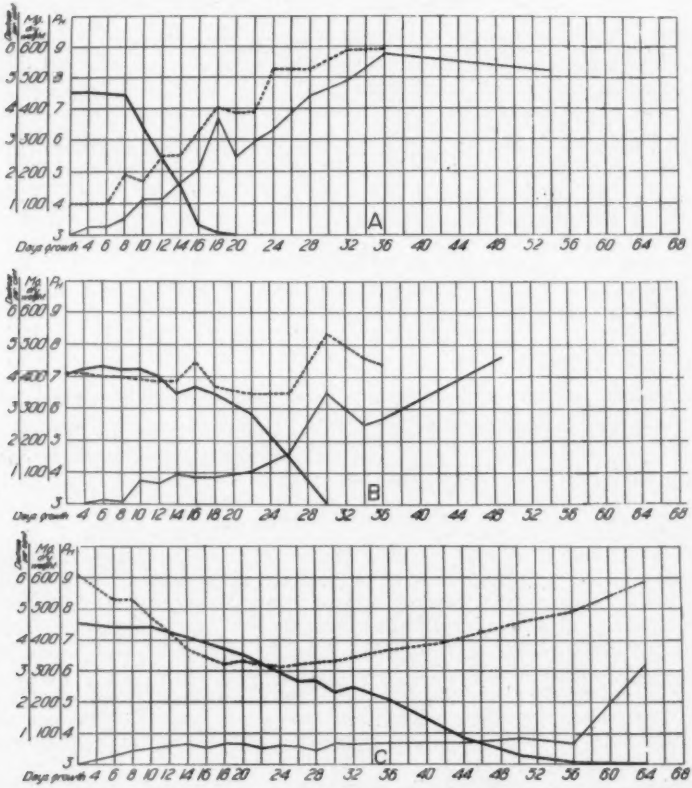
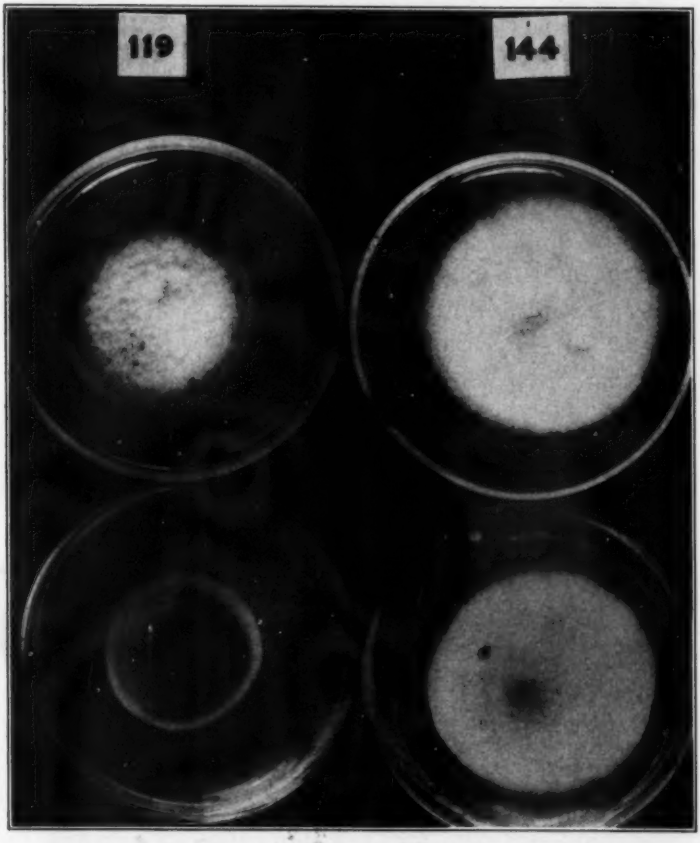


FIG. 8.—Dextrose utilization and changes in hydrogen-ion concentration as a result of the growth of *Fusarium lycopersici* on a modified Richards' solution. A, initial P_H, 4; B, initial P_H, 7; C, initial P_H, 9

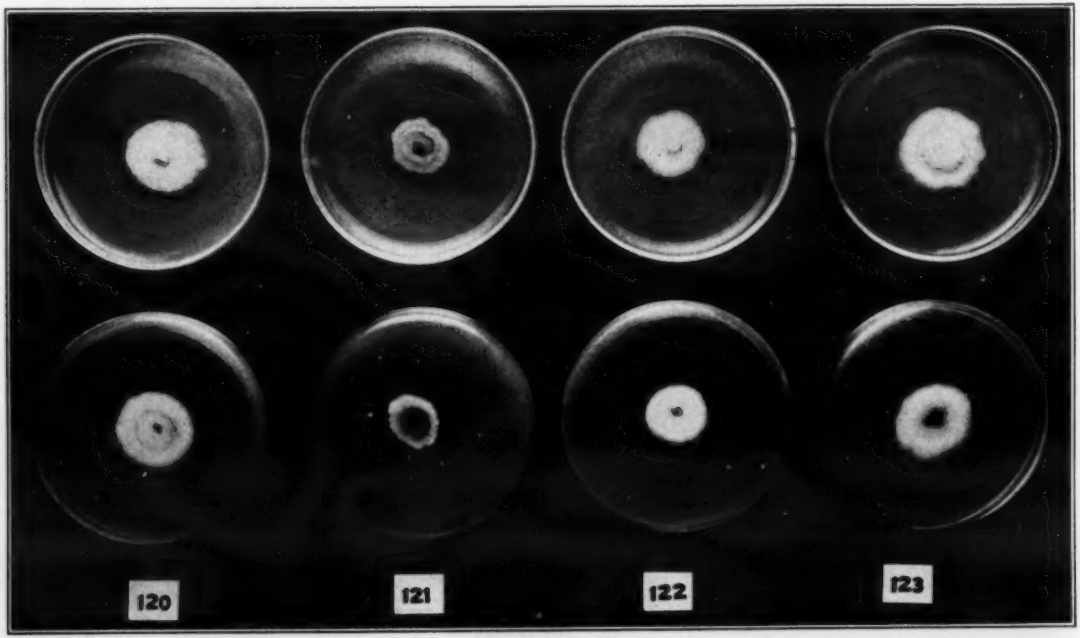
Discussion of Results

Utilization of dextrose as related to initial hydrogen-ion concentration.—With an initial P_H reading of 4± (series 1), dextrose was used rapidly, and at the end of 20 days traces only were left which disappeared in two more days. With an initial P_H of 7± (series 2), dextrose was used less rapidly, and measurable quantities were found at the end of 28 days, but none in 30 days. With an initial P_H of 9±, dextrose was used even less rapidly, and traces persisted after 51 days of growth.



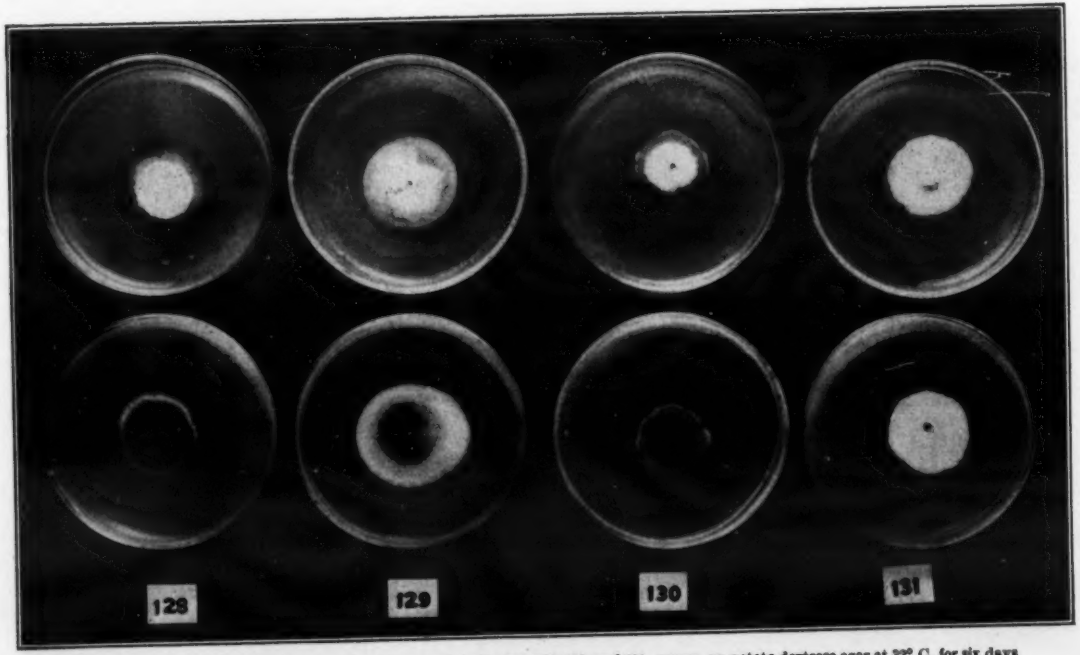
Surface (upper) and under side (lower) of culture of strain 119 (Iowa) and strain 144 (Wisconsin), grown on potato-dextrose agar at 20° C. for 10 days

Surface (upper) and under side (lower) of cultures of strains 60, 61, 68, and 119, grown on potato-dextrose agar at 32° C. for six days



Surface (upper) and under side (lower) of cultures of strains 120, 121, 122, and 123, grown on potato-dextrose agar at 32° C. for six days

Surface (upper) and under side (lower) of cultures of strains 124, 125, 126, and 127, grown on potato-dextrose agar at 32° C. for six days



Surface (upper) and under side (lower) of cultures of strains 128, 129, 130, and 131, grown on potato-dextrose agar at 32° C. for six days

Surface (upper) and under side (lower) of cultures of strains 132, 133, 134, and 143, grown on potato-dextrose agar at 32° C. for six days

Surface (upper) and under side (lower) of cultures of strains 144, 145, 140, and 150, grown on potato-dextrose agar at 32° C. for six days

TABLE 10.—*Dextrose utilization and hydrogen-ion changes as related to growth*

Number of days	Series 1, initial P_H reading, 4			Series 2, initial P_H reading, 7			Series 3, initial P_H reading, 9		
	Subsequent P_H readings	Dry weight of fungous mat in mgm.	Per cent of dextrose	Subsequent P_H readings	Dry weight of fungous mat in mgm.	Per cent of dextrose	Subsequent P_H readings	Dry weight of fungous mat in mgm.	Per cent of dextrose
0	4.04		4.50	7.15		4.14	9.04		4.54
4	4.04	28.1	4.54	7.174		4.27			
6	4.04	25.5	4.47	7.050	14.3	4.33	8.346	37.2	4.40
8	4.942	55.5	4.48	7.050	7.4	4.22	8.361	42.9	4.40
10	4.604	110.7	3.47	6.993	79.7	4.22	7.743	54.2	4.41
12	5.438	118.1	2.43	6.847	69.7	4.05			
14	5.528	164.1	1.54	6.847	95.6	3.54	6.712	67.1	4.10
16	6.261	210.2	.31	7.444	81.2	3.68	6.452	59.2	3.88
18	7.072	363.5	Trace.	6.689	81.6	3.52	6.295	65.4	3.69
20	6.813	242.2	.02				6.306	64.3	3.47
22	6.802	293.2		6.520	102.7	2.81	6.249	57.6	3.25
24	8.233	329.3					6.159	62.2	2.91
26				6.577	160.5	1.42	6.203	60.5	2.66
28	8.222	440.4					6.261	54.4	2.73
30				8.301	350.3		6.396	66.4	2.36
32	8.842	486.9					6.452	66.4	2.48
34				7.602	245.6				
36	8.865	572.8		7.410	267.7		6.666	72.2	2.09
44							6.982	70.5	.88
49					462.6				
51							7.579	81.6	.34
52		516.1							
56							7.872	67.9	Trace.
60							8.909	329.5	

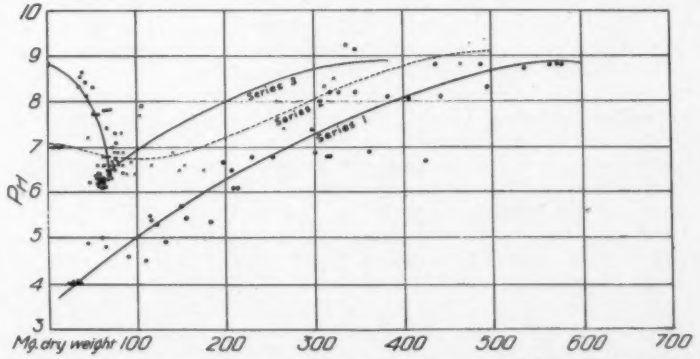


FIG. 9.—Relation of growth of *Fusarium lycopersici* to changes in the hydrogen-ion concentration of the solution

Correlated with this varied utilization of dextrose is the dry weight of the fungous mats produced. In series 1, growth was rapid from the start, after an initial lag due to the extremely small quantities of active mycelium present. Growth was less rapid in series 2, and the maximum dry weight obtained was never equal to that produced in series 1. Even more pronounced are these results in series 3. Here the dry weight produced even after long periods of growth was less than 100 mgm., the maximum being reached after about 14 days' growth. The remaining dextrose, from this time on, was apparently used only for respiration purposes, since no further increases in dry weight were recorded until after the dextrose had all been used.

The explanation of these variances seems to lie in the fact that organisms have a definite optimum hydrogen-ion concentration for growth. This optimum point was apparently reached early in the growth period of series 1. In series 2 and 3, if the optimum was ever reached, which is doutful, especially in series 3, it was reached later in the growth period, and after certain katabolic products had been formed which inhibited or retarded growth.

CHANGES IN HYDROGEN-ION CONCENTRATION CAUSED BY GROWTH.—Certain changes in the P_H readings of the solutions took place as a result of growth (fig. 8, A, B, and C). This phenomenon has been noted by many other investigators. Starting from an initial P_H of 4, the acidity of the culture solutions decreased rapidly until a P_H of 6.8 to 7 was reached. After a short time, during which all traces of dextrose disappeared, the acidity again continued to decrease until eventually a P_H of 8.8 was reached. Starting with an initial P_H of 7, only very slight changes took place in the acidity of the culture solutions until the sugar disappeared, when acidity decreased rapidly and a maximum P_H of 8.2 was reached. With an initial P_H of 9 the acidity was gradually increased as a result of growth until a minimum P_H of 6.1 to 6.2 was reached, after which the acidity gradually decreased. Between P_H 6.2 to 6.8 is the "isometabolic" point as defined by Sideris (*53*).

CONTINUED INCREASE IN DRY WEIGHT AFTER DISAPPEARANCE OF DEXTROSE.—In series 1 and 2 the fact is evident that after all traces of dextrose had disappeared from the culture solution, the fungous mat continued to increase in dry weight, without any apparent source of carbon. In series 1, for example, the dry weight at 36 days was more than twice that at 20 days, the last time dextrose could be detected in the solutions. It was thought that the dextrose might have been converted in this instance into salts of organic acids, alcohols, etc., which were subsequently used as sources of energy.

Analyses were therefore made of culture solutions 20 and 60 days old for salts of organic acids and alcohols. Both were found in larger amounts in the 20-day-old cultures than in the 60-day-old cultures, but the quantities were too small to account for the large increase in dry weight recorded for series 1. For example, salts of organic acids, as acetic, were found to be present as 0.00768 per cent in 20-day-old cultures, and 0.0024 per cent in 60-day-old cultures; the alcohols, 0.391 per cent by volume in 20-day-old cultures, and 0.0018 per cent in 60-day-old cultures.

In a duplicate series with Richards' solution, ethyl alcohol being used as the only source of carbon, 77.3 mgm. dry weight of *Fusarium lycopersici* were produced on a 0.5 per cent solution, the dry weight decreasing as the concentration of the alcohol either increased or decreased from this point. The continued production of dry matter by this organism after the sugar had disappeared could be explained in part, at least, by the conversion of some of the dextrose to ethyl alcohol and its subsequent use. Salts of organic acids probably play a less important part in this respect.

The thallus of 20-day-old cultures was very granular. The cells of the hyphae were filled with globules which gave the microchemical tests for glycogen only faintly. These globules of substance disappeared in older hyphae, and in cultures 60 days old could be found

only in the youngest hyphal tips. This suggested to the writer that the sugar as used was not all employed for the building up of protoplasm, but that a considerable part was stored in the cells for future use.

TOXIC EXCRETORY PRODUCTS PRODUCED BY FUSARIUM LYCOPERSICI, AND THEIR RELATION TO TOMATO WILT

INTRODUCTION

It has been the opinion of plant pathologists that fungi and bacteria causing the wilt diseases of various hosts produce wilting by mechanically plugging or otherwise obstructing the lumina of the fibrovascular bundles, thereby interfering with the free passage of water from the roots to the leaves (*12*, *22*, *36*, *41*, *42*, *54*). This conception of wilting is still found in literature published on the subject of wilts (*9*, *16*, *23*, *45*, *46*), and has much in its favor, especially since the appearance of the paper by Melhus et al. (*40*) upon this subject. Other explanations, however, have been advanced, chief among which are: (1) Decay of roots, causing decreased absorption, cutting the water supply down to a point where the host can no longer function normally; (2) the nutrition theory, which is based upon the supposition that the organisms growing within the lumen of the vessels deplete the liquids passing through of mineral elements necessary for normal growth of the host; (3) the toxic theory, based upon the supposition that the organisms concerned, as a result of growth, produce toxic excretory substances which cause the death of the host protoplasm.

It was noted that plants that had been infected with a wilt-producing pathogene often showed browned fibrovascular bundles from which the organism could not be isolated (*26*). It was further noted that the hyphae very rarely, if ever, were packed closely enough in the lumina to seriously hinder the passage of water (*5*, *57*). These and other facts were instrumental in the formulation of the theory that toxic excretory products were produced by the pathogene which were injurious to the cells of the host.

REVIEW OF LITERATURE

Haskell (*26*) has shown that decomposition products of the dead cells of the potato are able to discolor the fibrovascular bundles and are toxic to the cells with which they come into contact. He also showed that fibrovascular browning could be produced by the injection of certain toxic substances into the lumina of the bundles. Overton (*43*) and Dixon (*14*) concluded that the products of plant cells were toxic to the plants themselves, and that wilting might be due to the introduction of poisonous or plasmolyzing substances into the transpiration stream.

Tisdale (*57*), in his studies on flax wilt, has pointed out that partial destruction of the root system by the pathogene will interfere with the normal intake of water from the soil and cause a wilting under semidrought conditions. As early as 1892, Atkinson (*2*) pointed out that the fungus growing in the lumina of the fibrovascular bundles of cotton, and causing a wilt of that host, used a part of the food material which normally went to the host. This nutritional loss was thought to be a factor in the production of a wilted condition. The work

herein reported is concerned primarily with a study of the toxic excretory products produced by *Fusarium lycopersici*.

Brandes (*5*) was one of the first to demonstrate this toxic principle. He grew *Fusarium cubense* on Richards' and Uschinsky's solutions for two weeks. The cultures were then filtered aseptically. Cut stems of buckwheat plants, bean plants, and banana leaves were immersed in the filtrate, in sterile solution, and in distilled water. Wilting was found to be very rapid in those plants immersed in the filtrate, while the controls in sterile solution and distilled water remained turgid. He concluded from these experiments that wilting was not due to the plugging of the vessels, but was probably the result of toxic excretions by the fungus.

Fahmy (*18*), working on the toxic excretory products of *Fusarium solani*, concluded that the substance which was toxic and produced the wilting was thermostable and nonvolatile. Barnum (*4*) has shown that *Penicillium expansum*, classed by him as a strict saprophyte, is also capable of producing excretory substances which are toxic to plants. This substance or these substances are concluded to be thermostable and nonvolatile.

Picado (*44*) has made a study of the exo and endotoxins of *Fusarium solani*, *Verticillium albo-atrum*, *V. duboys*, and a saprophytic Penicillium. He concluded from his studies that wilting, browning of the vessels, and dissolution of tissue were not always due to the same cause. This conclusion is based upon the fact that wilting of plants was often more pronounced in heated extracts than in those not heated. This could not be the case if wilting were due entirely to substances of the nature of enzymes.

From previous studies on the toxic principles involved, it seemed to the author that probably two distinct toxic principles might be present. The earlier work on this subject indicates that the toxic substance is enzymic in nature. The later work of Fahmy (*18*) and Barnum (*4*), however, would lead one to believe otherwise, since they state that the toxic principle is thermostable, admittedly not a character of an enzyme.

The experiments reported below show that there are two such toxic principles involved. One is enzymic and therefore thermolabile; the other is crystalloidal and thermostable.

EXPERIMENTAL METHODS

In these experiments strain 134 was used. The organism was pure lined by the usual methods and was kept in culture on potato-dextrose hard agar with frequent transfers from the time it was isolated until it was used. This strain was found to be strongly pathogenic to susceptible varieties of tomatoes in the greenhouse.

The culture solution chosen was a modification of Richards' solution. Brandes (*5*) suggested that when sucrose was used as a source of carbon for fungi, it was broken down to glucose and fructose before being used by the organism. One molecule of sucrose thus gives rise to one molecule each of glucose and fructose. This would double the osmotic pressure of the solution if complete reduction took place and if none of the glucose or fructose were used in the metabolism of the organism. In any case, the osmotic pressure of the solution after growth of the organism upon it, would be increased. This

increase might be large enough in some cases to cause the osmotic pressure of the filtrate to be greater than that of the cut stems immersed in it. If such a condition existed, water would pass out of the cells of the immersed stems and wilting would result, which in no way would be due to the presence of toxic products in the solution. To obviate this difficulty, Brandes used Uschinsky's solution, in which the carbon source is supplied by small quantities of sodium asparaginate, ammonium lactate, and glycerine. This solution was tried by the writer for *Fusarium lycopersici*, but this organism grew very poorly on it. As a result Richards'[11] solution was used, the sucrose being replaced by glucose. Brannon (*6*) has shown that both glucose and fructose are used somewhat similarly in growth, and that there is no choice between them as sources of carbon supply for fungous nutrition. *Fusarium lycopersici* made a rapid growth upon this medium and after three weeks' time, the osmotic pressure of the filtrate was found to be practically the same as that of sterile solution.

Richards' solution, 250 c. c., was placed in liter Erlenmeyer flasks and sterilized in the autoclave at 15 pounds pressure for 20 minutes. The flasks were incubated for two days, after which they were inoculated with 1 c. c. of a heavy suspension of microconidia of the organism. At the end of two weeks at ordinary room temperature a heavy mycelial mat had formed. The cultures were filtered through a Büchner funnel, and the filtrate, an amber-colored solution, was used in these experiments.

EXPERIMENTAL RESULTS

These experiments were carried on in the greenhouse on sunny days, except where otherwise noted. The temperatures of the greenhouse fluctuated between 75 and 85 °F.

EXPERIMENT 1.—TESTS ON THE TOXICITY OF THE FILTRATE TO CUT TOMATO STEMS

In order to test for toxic substances, the filtrate from a 4-weeks-old culture was poured into 250 c. c. wide-mouthed bottles. Young tomato plants of the susceptible variety John Baer were cut under water and immersed at once in the filtrate. As controls, similar plants were immersed in sterile Richards' solution and in distilled water. The filtrate and the sterile solutions were used undiluted or tap water was added in such quantities as to give dilutions of 50 and 75 per cent. This was done in order to avoid high osmotic pressures which might cause wilting. In this and in other experiments it was found, however, that this was a needless precaution, since plants immersed in the pure sterile solution failed to wilt after long periods of time, but remained turgid and made growth.

At the end of one hour the plant in the 100 per cent filtrate was badly wilted. At the end of seven hours all the plants in the filtrate at both dilutions were very badly wilted, the less the dilution the greater the degree of wilting. Those in sterile Richards' solution were all turgid, as were the plants in distilled water. Plate 8, A and B, shows the condition of these plants at the end of 24 hours.

[11] Hereafter in this paper when Richards's solution is mentioned, the modification described here is also assumed, unless otherwise noted. KNO_3, 10 gm.; KH_2PO_4, 5 gm.; $MgSO_4$, 2.5 gm.; $FeCl_3$, 20 mgm.; dextrose, 50 gm.; water 1,000 c. c.

The wilting caused by the toxic substances in the filtrate was rapid and destructive. The leaves became dark green with a water-soaked appearance and within 24 hours were dead and dry.

Cabbage plants 10 weeks old with four to six leaves were immersed in these same solutions. Those in the pure filtrate all wilted within eight hours, the most severe wilting occurring in the pure filtrate. Those in sterile Richards' solution were turgid after 48 hours.

EXPERIMENT 2.—TESTS ON THE TOXICITY OF THE FILTRATE TO TOMATO PLANTS GROWN IN WATER CULTURE

This experiment was designed to test the effect of the filtrate upon growing plants whose roots had not been injured. Plants of the John Baer variety were used. The seed was sown in clean sand, and when the plants had produced their first true leaves they were transferred to water cultures. Wide-mouthed bottles having a capacity of 250 c. c., covered with black paper and sealed over the top with paraffined paper, were used. In the center of the cap a small opening was made for the stem of the young plant, and near the edge a larger opening was made for the introduction of the culture solution. The plants were grown for eight weeks in Knop's solution. They made a vigorous growth and developed an extensive root system. At the end of the eight-week period the Knop's solution was siphoned off and was immediately replaced by the filtrate from a 4-weeks-old culture of the organism on Richards' solution. Dilutions were made as in experiment 1. Controls consisted of plants in sterile Richards' solution and distilled water. The plants in the solutions wilted at once, most likely because of the plasmolyzing of the root cells which came in contact with the solutions. The plants in distilled water did not wilt. All plants had recovered in four hours.

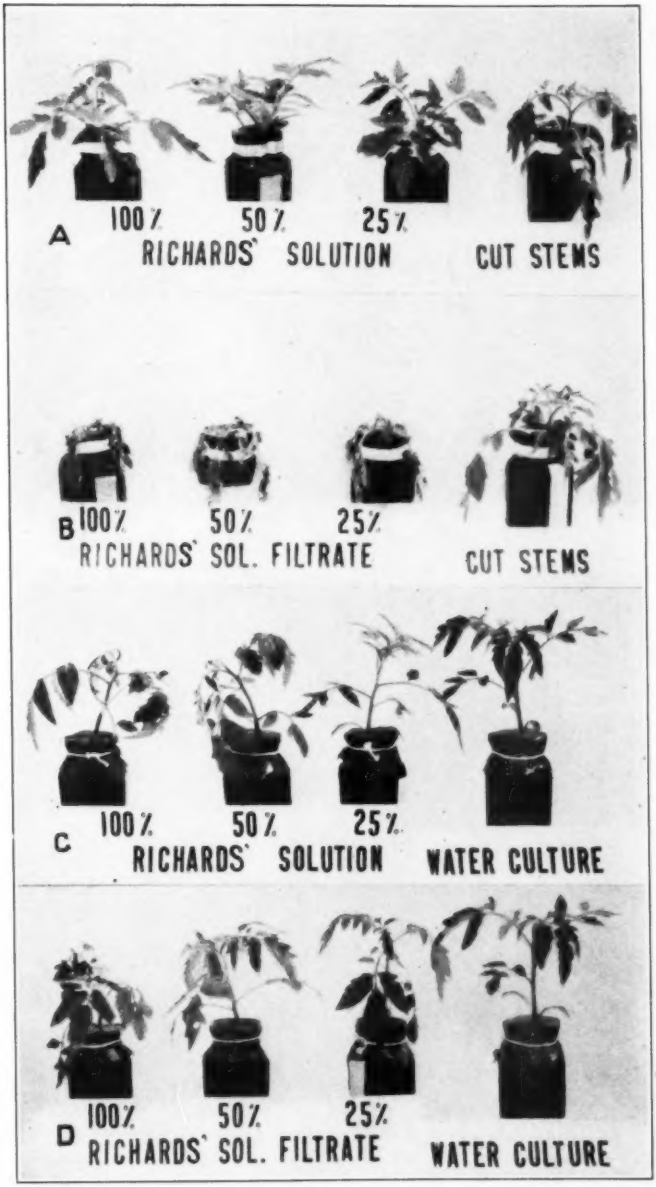
At the end of 24 hours all the plants were turgid and growing, having recovered from the wilting caused by the sudden change from a solution of lower osmotic pressure to one of higher. The condition of these plants at the end of 24 hours is shown in Plate 8, C and D.

The roots of the plants growing in the filtrate were examined after 48 hours, and were found to be normal in every respect and similar to those in the sterile solution and the distilled water.

EXPERIMENT 3.—EXTRACTION AND TESTS FOR TOXICITY OF INTRA- AND EXTRA-CELLULAR SUBSTANCES

That some toxic excretory material is present in the filtrate is evident from experiment 1. Since it apparently failed to diffuse through the semipermeable membranes of the root hairs, its colloidal nature seemed evident. This experiment was concerned with the separation of the intra- and extra-cellular enzymes produced by the organism and the testing of water solutions of the enzymes obtained to determine their ability to bring about wilting of cut tomato stems.

A 2-weeks-old culture of the organism was filtered in the usual way. The mycelial mat was left in 20 per cent alcohol for 24 hours and the enzymes extracted were precipitated with 95 per cent alcohol. The precipitate was filtered off on filter paper and dried. It was redissolved in 600 c. c. of distilled water. This enzyme solution

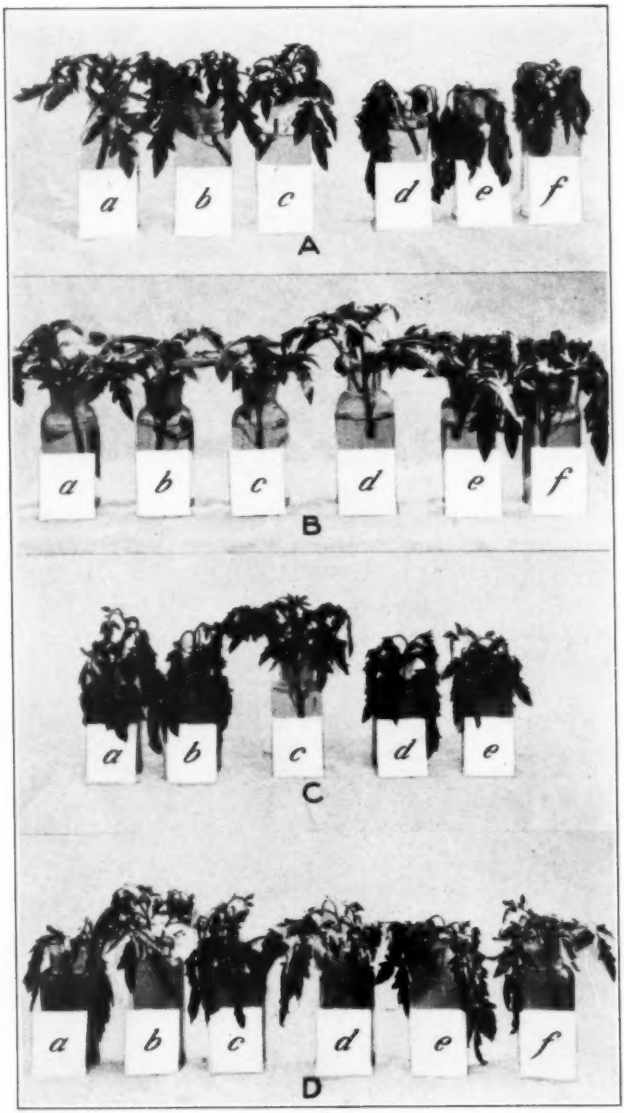


A, cut stems of John Baer tomato plants immersed in sterile Richards' solution with the indicated dilutions; plant on right, control in distilled water; photographed after 24 hours.

B, cut stems of John Baer tomato plants immersed in the filtrate from a 4-weeks-old culture of *Fusarium lycopersici* on Richards' solution, with the indicated dilutions; plant on right, control in distilled water; photographed after 24 hours.

C, John Baer plants grown in Knop's solution for eight weeks, Knop's solution then replaced by sterile Richards' solution with the indicated dilutions; plant on right, control in distilled water; photographed after 24 hours.

D, same as C, except that Knop's solution was replaced by the filtrate from a 4-weeks-old culture of *Fusarium lycopersici* on Richards' solution with the indicated dilutions; plant on right, control in distilled water.



A, *a*, *b*, *c*, cut tomato stems of John Baer plants immersed in distilled water; *d*, *e*, *f*, John Baer plants immersed in distilled water solutions of the intracellular enzymes extracted from a mycelial mat of *Fusarium lycopersici*; photographed after five hours.

B. Treatment of plants similar to that in A except that enzyme solution used was boiled for 10 minutes; photographed after 24 hours.

C, *a*, *b*, cut tomato stems of John Baer plants immersed in dialyzate; *d*, *e*, in diffusate from a 2-weeks-old culture of *Fusarium lycopersici* on Richards' solution; *c*, control in sterile Richards' solution diluted to 50 per cent of its original concentration; photographed after 30 hours.

D, Repetition of experiment shown in C; *a*, *b*, *c*, in dialyzate; *d*, *e*, *f*, in diffusate; photographed after 50 hours.

was divided into three 200 c. c. lots and placed in wide-mouthed bottles. Young vigorous tomato plants were cut under water and immersed immediately in the solutions. Three similar plants were immersed in distilled water and used as controls.

At the end of five hours the plants immersed in the enzyme solutions were badly wilted, while those in distilled water were turgid (pl. 9, A). The enzyme solution was removed and boiled for 10 minutes. A flocculent grayish-white precipitate resulted. The boiled enzyme solution was again divided into three 200 c. c. lots and placed in wide-mouthed bottles as before. Young tomato plants were immersed as before, both in the solutions and in the distilled water. All the plants remained turgid and showed no signs of wilting at the end of 24 hours (pl. 9, B). The toxic properties of the substances extracted from the mycelial mat are evident, and have properties similar to those possessed by enzymes. Exactly similar results were obtained when the enzymes contained in the filtrate were precipitated with 95 per cent alcohol and used in water solutions.

Several plants of the Black Eyebrow soybean and Early Buff cowpea were immersed in both the intra- and extra-cellular enzyme solutions used above. When examined at the end of two hours, the soybeans were very badly wilted, while the cowpeas were only slightly wilted.

Experiment 4.—Test of Boiled and Unboiled Filtrate

From the preceding experiments it appeared that the toxic properties of the culture solutions upon which *Fusarium lycopersici* had been grown were due to enzymes secreted by the fungus. It seemed, therefore, that this toxic property if entirely enzymic in nature could be completely destroyed by boiling the filtrate. Consequently, the filtrate from a 4-weeks-old culture was divided into two portions. Each portion was divided into three parts. One part of each was kept at its original strength, a second part diluted to half its original strength with distilled water, and the third part diluted to one quarter of its original strength with distilled water.

The three parts of one portion were now boiled for 15 minutes. The solutions were placed in 250 c. c. wide-mouthed bottles, and stems of young, vigorous tomato plants, cut under water, were immersed in them. As a control, similar plants were immersed in sterile Richards' solution. In five hours all the plants in both the boiled and unboiled filtrate were badly wilted. The control plants remained turgid for 24 hours. Boiling the filtrate did not deactivate the toxic principle, a fact which led the writer to suspect that a thermostable substance toxic to tomato plants was also present in the filtrate. The substance could not be enzymic in nature, since enzymes are destroyed by boiling. The possibility suggested itself that the thermostable substance was crystalloidal in character. The following experiment was conducted with this idea in mind.

Experiment 5.—Toxicity of Dialyzed Filtrate

A 2-weeks-old culture of *Fusarium lycopersici* was filtered in the usual manner through a Büchner funnel. Three cultures produced 750 c. c. of filtrate. This was placed in a dialyzer made of two thicknesses of parchment paper. The filtrate was covered with

a thin film of toluene to prevent contamination. This dialyzer was suspended in 750 c. c. of distilled water. At the end of 60 hours, the diffusate was amber colored as was the dialyzate. Because of the higher osmotic pressure of the filtrate, water passed into the parchment bag, so that at the end of 60 hours only 600 c. c. of the diffusate was recoverable, while the dialyzate had increased in volume to 900 c. c. These were siphoned off into 250 c. c. wide-mouthed bottles, and cut stems of young vigorous tomato plants of a susceptible variety were immersed in them at once. Controls consisted of similar plants immersed in sterile Richards' solution diluted to 50 per cent of its original strength. This dilution was made in order that approximately the same osmotic pressure relationships would exist over the whole series. Wilting was slight in all plants within five hours, with the exception of the controls. The condition of the plants in this series at the end of 30 hours is shown in Plate 9, C. Plants in both the diffusate and dialyzate were equally wilted at the termination of this time. The solutions were subjected to 15 minutes boiling, after which freshly cut stems of tomato plants were immersed as before. Wilting occurred as above, both in the diffusate and dialyzate, although not so rapidly as before boiling. This experiment was repeated with similar results (pl. 9, D).

EXPERIMENT 6.—OSMOTIC PRESSURES IN RELATION TO TOXICITY OF SOLUTIONS USED IN PREVIOUS EXPERIMENTS

It was deemed advisable at this stage of the studies to repeat the work already done in its entirety, before further work was attempted. Consequently, freshly cut, 6-weeks-old tomato plants of the John Baer variety were immersed in the following solutions: Distilled water, tap water, distilled water plus the extracellular enzymes from the filtrate of a 3-weeks-old culture of *Fusarium lycopersici*, distilled water plus the intracellular enzymes obtained from the mycelial mats of two 3-weeks-old cultures of *F. lycopersici*, pure filtrate from a 3-weeks-old culture; filtrate diluted 50 per cent with distilled water, filtrate diluted 75 per cent with distilled water, diffusate from two 3-weeks-old cultures, dialyzate from the same source, pure sterile Richards' solution, sterile Richards' solution diluted 50 per cent with distilled water, and sterile Richards' solution diluted 75 per cent with distilled water.

The osmotic pressures of all the solutions except the tap and distilled waters were determined cryoscopically with the aid of a Beckmann freezing apparatus. Notes were taken at intervals upon the behavior and appearance of the plants. The data obtained appear in tabular form as Table 11. It was unfortunate for the experiment that the day the solutions were ready to use was cloudy. Bright days induce rapid transpiration and, therefore, a rapid rise of the toxic principles into the plant parts through the transpiration stream and result in bringing about a more rapid wilting. However, the results were satisfactory and confirmed those previously obtained.

TABLE 11.—*Behavior of 6-weeks-old John Baer tomato plants when cut and immersed in various solutions*

Solution	Osmotic pressure in atmospheres	General appearance of the plants after—		
		2 hours	4 hours	24 hours
Distilled water	0.0	Turgid	Turgid	Turgid.
Tap water		do	do	Do.
Distilled water plus extracellular enzymes from filtrate of F. lycopersici culture.	1.44	Wilted	Badly wilted	Very badly wilted.
Distilled water plus intracellular enzymes from mycelial mats.	.48	Badly wilted	Dead	Dead.
Pure filtrate	11.68	Turgid	Wilted	Do.
Filtrate diluted 50 per cent with distilled water.	5.90	do	Slightly wilted	Very badly wilted
Filtrate diluted 75 per cent with distilled water.	3.13	do	Turgid	Do.
Diffusate	6.38	do	do	Badly wilted.
Dialyzate	5.05	do	do	Do.
Pure sterile Richards' solution	12.16	do	do	Turgid.
Sterile Richards' solution diluted 50 per cent with distilled water.	6.02	do	do	Do.
Sterile Richards' solution diluted 75 per cent with distilled water.	2.41	do	do	Do.

It should be explained that after 24 hours in the extracellular enzyme solution, the bundles were browned for 6 inches into the stem. In the case of the intracellular enzyme solutions, the bundles of the plants immersed were also browned and the leaves were dry and brittle. Attempts were made to isolate an organism from these browned fibrovascular bundles, but the results were negative. The stems of plants immersed in the dialyzate and all dilutions of the filtrate were watersoaked and slimy in appearance as contrasted with the shriveled but firm stems of the plants immersed in the diffusate. After 24 hours all these plants were very badly wilted.

These experiments were repeated in part with a new set of solutions with very similar results. The plants immersed in distilled water and in sterile Richards' solution remained turgid for 48 hours, while those immersed in the enzyme solutions were badly wilted at the end of three hours and were dead and dry at the end of 30 hours. The plants immersed in the dialyzate and diffusate from a 3-weeks-old culture were equally wilted at the end of 30 hours, but the plants in the dialyzate wilted sooner than those in the diffusate. Those plants immersed in the filtrate were badly wilted at the end of 30 hours, those in the pure filtrate being dead and dry at the end of that time.

EXPERIMENT 7.—TOXICITY OF FILTRATES TO RESISTANT AND SUSCEPTIBLE VARIETIES

This experiment was designed to test the effects of the filtrate from a 3-weeks-old culture of *Fusarium lycopersici* grown on Uschinsky's solution on susceptible and resistant varieties of tomatoes. Three wilt-producing organisms were used: *F. lycopersici* from St. John, Kans., strain 134; *F. lycopersici*, from Manhattan, Kans., strain 133; and *F. oxysporum* Schlecht, obtained from G. B. Ramsey. At the end of three weeks' growth (which was very poor) of these organisms on Uschinsky's solution, the cultures were filtered in the usual manner and the filtrate placed in pint fruit jars. As a check upon

the behavior of the plants immersed in the filtrates, plants were immersed in sterile Uschinsky's solution. Young tomato plants, 8 to 10 inches in height, of the varieties Comet and Kanora were used. The former variety is susceptible, while the latter is highly resistant. The young plants were grown in pots and were cut under water and transferred at once to the filtrates, three plants being placed in each jar. The results obtained are shown in Table 12.

At the end of one hour wilting was evident in all the plants except the controls. The Comet plants were more wilted than the Kanora, although all are classed as slightly wilted in the table. At the end of two hours, the Comets in the filtrate from the culture of *F. oxysporum* were badly wilted, while the Kanora plants were still only slightly wilted. At the end of six hours the difference in wilting between plants of the two varieties was even more evident. The resistant Kanora plants still showed slight wilting; the susceptible Comets were badly wilted in the filtrates of *F. lycopersici*, and very badly wilted in the filtrate from *F. oxysporum*.

TABLE 12.—*Behavior of Comet and Kanora tomato plants immersed in filtrates from cultures of wilt-producing Fusaria*

Solution	Variety	Appearance of plants after—		
		1 hour	2 hours	6 hours
Sterile Uschinsky's solution	Kanora	Turgid	Turgid	Turgid.
	Comet	do	do	Do.
Filtrate No. 133	Kanora	Slightly wilted	Slightly wilted	Slightly wilted.
	Comet	do	do	Badly wilted.
Filtrate No. 134	Kanora	do	do	Slightly wilted.
	Comet	do	do	Badly wilted.
Filtrate *F. oxysporum*	Kanora	do	do	Slightly wilted.
	Comet	do	Badly wilted	Very badly wilted.

EXPERIMENT 8.—DISTILLATION OF FILTRATES

To determine the volatility of the nonenzymic toxic substance or substances, distillation of the filtrate was carried out. One liter of filtrate from a 6-weeks-old culture of the organism on Richards' solution was fractionally distilled. Eight 100 c. c. fractions were collected, and cut stems of tomato plants of the John Baer variety were immersed in them. The first fraction proved highly toxic, wilting of the plants immersed in it occurring within two hours. The second fraction was much less toxic and the remaining six fractions were nontoxic. The residue was highly toxic. Evidently both volatile and nonvolatile substances are present which are toxic to cut tomato plants.

EXPERIMENT 9.—TESTS FOR TOXICITY ON CUT TOMATO STEMS OF SUBSTANCES ISOLATED FROM SOLUTIONS UPON WHICH FUSARIUM LYCOPERSICI HAD BEEN GROWN

It has previously been pointed out in this paper (p. 222) that solutions upon which *Fusarium lycopersici* were grown, contained varying amounts of alcohol and salts of organic acids. In order to determine if these substances were toxic to cut tomato stems, and were possibly concerned in the wilting of tomatoes infected with this organism,

solutions of organic acids and ethyl alcohol were prepared in distilled water. Cut tomato stems were immersed in these solutions and their behavior noted.

Ethyl alcohol was not toxic to cut tomato stems in concentrations up to 4 per cent. Higher concentrations were not used. The plants immersed in alcohol solutions remained turgid, and after four days in the solutions showed no ill effects. During this time large quantities of the solutions had been transpired.

The organic acids used were acetic, proprionic, normal butyric, and normal valeric, of the fatty-acid series; and lactic, oxalic, tartaric, and benzoic. All of these acids proved extremely toxic to cut tomato plants. All except lactic and tartaric acids produced rapid wilting and death of the plants at as low concentrations as 0.06 of 1 per cent. At this concentration lactic and tartaric acids produced slight wilting only at the end of four hours, while the other acids had caused death of the seedlings at this concentration in a much shorter time. Lactic and tartaric acids were highly toxic at 0.12 of 1 per cent concentration. The cells of the stems of plants immersed in oxalic acid were disintegrated. None of the other acids had this effect. Benzoic acid bleached the stems of all color.

It is clear that these acids in very low concentrations are highly toxic to tomato plants, and may be a factor in the wilting of plants caused by vascular parasites.

DISCUSSION

From the foregoing experiments, it is evident that culture solutions upon which *Fusarium lycopersici* has grown are toxic to tomato plants. Some light has been thrown upon the nature of this toxic principle by a study of some of its properties. It has been found that there are at least two substances or groups of substances with certain opposite characters capable of causing wilting in tomato plants. Whether or not the same substances are elaborated within the host as are elaborated by the fungus when grown on artificial culture media, has not been investigated. The action of the organism upon the cells of the host may result in the production of toxic substances entirely distinct from those produced by the organism when grown unassociated with the host. That products of dead plant cells may be toxic to the plant itself has been shown by Haskell (*26*). However, the experiments on the wilting of susceptible and resistant varieties of tomatoes when immersed in filtrates of cultures of *F. lycopersici* would indicate that plants of the variety Kanora, resistant to wilt under field conditions, are also more resistant to the toxic substances elaborated by the fungus in pure culture than are plants of the susceptible variety Comet. Resistance may possibly be due, therefore, to substances within the host protoplasm which offset or counteract the injurious effects of the toxic excretory products of the pathogene.

These toxic excretory products fall into two categories; one of which has been determined to be colloidal in nature, nondialyzable, and thermolabile. In all probability this is of the nature of an enzyme and is both extra- and intra-cellular in character. From the experiments on dialysis of the filtrate, it was found that the diffusate was also capable of bringing about rapid wilting and death of cut

tomato plants when immersed in it. Boiling either the dialyzate or diffusate did not deactivate the toxicity of the solutions. That there is present a thermostable toxic substance, crystalloidal in nature, is also proved by the experiment with boiled and unboiled filtrate. That this crystalloidal substance is not of the nature of a coenzyme is evident from the dialyzing experiments. When separated from the nondialyzable portion of the enzyme, coenzymes lose their enzymic properties. Therefore, the diffusate of these experiments should be nontoxic if the dialyzable substance or substances were coenzymic in nature. Since this was not the case, we are led to assume that these two toxic principles are distinct from each other. We would expect, therefore, that the plants that had been grown in water culture and whose roots were then bathed in the filtrate from an old culture of *Fusarium lycopersici* would wilt as a result of the diffusion into the roots of the crystalloidal toxic substance. Barnum (*4*) obtained wilting of cabbage plants whose roots he claimed to be uninjured when immersed in filtrates from cultures of *Penicillium expansum*. It seems to the writer, however, doubtful whether a young plant growing in sand could be removed without breaking some of the roots. Mix [12] obtained wilting when plants grown in water culture were transferred to filtrates of a number of organisms.

In the light of the experiments reported herein the writer is unable to explain why the plants grown in Knop's solution in experiment 2 and then transferred to a filtrate from an old culture of *F. lycopersici* did not wilt.

No attempt has been made to identify the enzymes extracted. Some of the substances present in the filtrate in true solution have already been mentioned. Lathrop (*32*) has shown that *Fusarium cubense* EFS. produces a volatile aldehyde when grown on Uschinsky's solution. He suggests that this aldehyde may be toxic. Volatile substances are also concerned in the case of tomato wilt, since the first two 100 c. c. fractions of distillation proved toxic to cut tomato plants. This is in agreement with the results of Fahmy (*18*) on *F. solani* Ap. et Wr., who also demonstrated the presence of ammonia and of an oxalate in the filtrate from his cultures. These substances were found in small quantities only, and were not concentrated enough to cause the wilting which he observed.

Of the substances found in true solution in liquid cultures upon which *Fusarium lycopersici* has been grown, alcohol was proved to be nontoxic to tomato plants. Organic acids, however, salts of which are present, were proved to be extremely toxic in low concentration. The extreme toxicity of some of these organic acids in concentrations of 0.06 of 1 per cent over short periods of time would indicate their toxicity in still lower concentrations over longer periods of time. Whether or not these acids are formed in the normal relationships between the host and parasite is unknown, and has not been investigated.

The toxic substances demonstrated in these experiments are evidently nonspecific in character. Those produced by the growth of *Fusarium lycopersici* on Richards' solution were found to cause wilting of cowpeas, soy beans, and cabbages as well as tomatoes. The filtrate from a culture of *F. oxysporum* was found to be more

[12] A. J. Mix, University of Kansas, in conversation with the author.

toxic to cut tomato plants than was the filtrate from cultures of two strains of *F. lycopersici*. It seems probable that many fungi during their growth secrete growth products toxic to plants if present in the transpiration stream. As yet the rôle of these toxic excretory products in the production of a diseased condition is little understood, and the nature of these products even less so. It is the belief of the writer that the actual damage caused by the presence of the fungus in the lumina of the vascular bundles has been overestimated, and the injury done by toxic substances produced by the organism and diffusing into the transpiration stream underestimated. In other words, the systemic invasion of a host by a wilt-producing organism would be of less importance if the toxic substances produced were either absent or counteracted.

It seems likely that the resistance of certain tomato varieties to wilt may be due in part to the presence of substances within the host protoplasm capable of uniting with or adsorbing the enzymes or toxins elaborated by the parasite, and thus annulling their destructive action. That we have been unable to develop varieties of tomatoes immune to wilt is evidence in support of this theory. Resistant varieties, theoretically, possess substances within the protoplasm which counteract the destructive influence of the enzymes or toxins secreted by the parasite and upon which the organism depends for its parasitic habit. However, these substances may have no effect upon the crystalloidal, diffusable toxic substance which has also been shown to cause wilt of tomato plants. It is for such wilt-producing substances that Picado (*44*) would search for antidotes. Past experience on the injection of substances into hosts to produce a condition of immunity to plant diseases has been on the whole unsatisfactory. Before an antidote could be intelligently prepared for these crystalloidal toxic substances, their chemical nature should be fully understood. Future investigations should be conducted along these lines, so that the nature of resistance and susceptibility to tomato wilt may be more fully understood.

GENERAL CONSIDERATIONS

The results obtained by the study of 24 isolations of Fusaria from wilted tomato plants have emphasized the fact that isolations of the same organism from widely different regions or localities may be expected to differ in virulence and in certain other physiological characters. Only in a general way have the different degrees of pathogenicity been correlated with temperature relations and growth rates, and these facts could not be correlated with very evident differences expressed by the strains toward the hydrogen-ion concentrations of the substrate upon which they were being grown. The statement of McClintock (*35*) and others, however, that strains of tomatoes resistant to wilt in one locality are not always adapted to other localities, has some additional experimental evidence in its support. That this might be due to differences in the pathogene rather than to differences in the environment was suspected by Edgerton (*15*) and Clayton (*11*), who found that strains of the pathogene from different localities reacted differently toward tomato varieties. That such strains vary in at least two physiological characters has been shown by these studies.

By a study of the temperature relations and growth rates, the 24 strains were divisible into two groups; one group with a wider temperature range and more rapid growth rates, consisting of five members, one each from New York, Wisconsin, and Texas, and two from Louisiana, and the other group of 19 members with a more limited temperature range and slower growth rates. The wider temperature ranges and more rapid growth rates expressed by the first of these groups was correlated with a higher degree of virulence than that expressed by the members of the other group. It was further correlated with certain cultural characters as described in the text.

By a study of the hydrogen-ion-growth curves, differences in the behavior of the several strains were noted, but it was impossible to group them or to correlate these differences with the differences found in virulence, growth rates, temperature relations, or cultural characters, as far as these were studied. Nine strains were noted, however, which evidently failed to use dextrose as efficiently as a carbon source as the remaining 15 strains, judging from comparative dry weights produced over a 10-day period on a liquid medium.

Scott (*49*) has expressed the opinion that soils might be adjusted in their reaction so that they would show a hydrogen-ion concentration equal to that at which the minimum of growth of the organism and consequently the minimum of infection occurs. While he was unable to accomplish this in one field experiment owing to the fact that his soil treatments did not reach the depth to which the roots of the plants penetrated, Hopkins (*28*) was able to accomplish it with soils in the greenhouse inoculated with *Gibberella saubinetii* and on which wheat was grown. Provided the hydrogen-ion growth curve of the organism in question has been determined, this is theoretically, if not practically, a method of partial control of the disease under investigation. However, without the hydrogen-ion growth curve of the organism involved, this method may be as much of a danger as a possible benefit. For example, the minimum point in the growth curve of strain 128 in these experiments (fig. 6, A) occurs at P_H 5.6 to 6.0. The maximum of strain 129 occurs at the same range. To adjust the soil at the minimum point for one strain would be to adjust it at the maximum point for the other.

In this connection, the results of the pathogenicity studies reported in this paper may be questioned. The reaction of the soil used was not determined, and it is quite likely that the soil reaction permitted a maximum growth of some of the strains, and consequently a maximum of infection and disease, while it allowed a minimum of growth with a minimum of infection and disease for other strains. The comparative virulence of the strains might have been more accurately determined by conducting such tests at the optimum points for each of the several strains. Had this been done the several strains might have appeared equally virulent. However, it should be mentioned that the five most virulent strains, namely, 120, 122, 127, 131, and 144, showed in their hydrogen-ion growth curves different maximum points.

The fact that certain substances toxic to the hosts are produced by pathogenes and nonpathogenes when grown in pure culture, is nothing new. Whether these pathogenes produce the same or even similar toxic substance when in relation to their hosts is questionable

and as yet unproved. Further study upon this subject might profitably be directed to a study of the chemical relations between the host and parasite.

SUMMARY

The temperature relations, growth rates, and hydrogen-ion relations of 24 strains of Fusaria isolated from wilted tomato plants from various parts of the United States were studied.

The strains showed different degrees of virulence toward seedling tomato plants in greenhouse tests.

The 5 strains, Nos. 120, 122, 127, 131, and 144, were found to be especially virulent in their attack upon tomato seedlings in these greenhouse tests, and markedly more destructive than the remaining 19 strain. They were classed as a group, known as the 120 group. The other strains were classed as a second group known as the 60 group.

Strains in the 120 group exhibited slightly wider temperature ranges and more rapid growth rates than the strains in the 60 group.

Strains in the 120 group differed from the strains in the 60 group in color production on potato-dextrose agar and in the production of macroconidia on wheat kernels, but were similar to the strains in the 60 group when grown on steamed rice, steamed potato plugs, steamed tomato stems, and potato agar.

The strains used in these experiments were found to be of the nonstaling type of fungi when grown on potato-dextrose agar, but were of the staling type when grown on liquid synthetic media.

It was found in determining the mean daily increment increases of the mycelial disks that a 4, 5, 6, or 7 day growth period could be used from which to obtain the mean.

The hydrogen-ion relations of the 24 strains were determined. In general the hydrogen-ion growth curves were similar to each other.

Two maximum growth points in the curves were evident. The first maximum point was rather consistent in its position upon the scale, but the minimum growth point, the second maximum, and the limit of alkalinity tolerated were found to be more or less variable.

The limits of acidity and alkalinity tolerated were determined approximately as P_H 2.2 to 3.1 and P_H 8.6 to 10.4, varying with the strain. The first maximum appeared at a range of 4.5 to 5.5, the minimum in the curve usually appeared at 6 to 6.5, and the second maximum usually above P_H 7, but in three cases appeared just below neutrality.

Four strains showed their second maximum point the larger of the two. In one of these cases the second maximum occurred at P_H 8.8, while the other three showed their second maximum at or just below neutrality.

Nine strains used dextrose as a source of carbon less efficiently than the remaining 15. All strains grew very poorly on Uschinsky's solution, in which the carbon source is supplied as small quantities of sodium asparaginate, ammonium lactate, and glycerine.

When *Fusarium lycopersici* is grown on Richards' solution, with a P_H of 4, the acidity is decreased until a P_H of 8.8 is reached, after which no more growth takes place and no further decreases of acidity are noted. When it is grown on Richards' solution with an initial

acidity of P_H 7, slight increases in acidity occur up to the time the sugar disappears, after which acidity is decreased rapidly to P_H 8+. When it is grown on Richards' solution with an initial acidity of P_H 9, increases in acidity take place with little growth until a minimum P_H of 6.1 to 6.2 is reached. This acidity remains constant until after the sugar disappears, when acidity is decreased rapidly, reaching a final P_H of 8+. The higher the initial P_H within the limits noted, the slower is growth and sugar utilization.

When *Fusarium lycopersici* is grown on a modified Richards' solution it produces substances which are highly toxic to cut tomato plants, causing them to wilt and die in a short time.

These toxic substances have been separated by physicochemical means into two classes: The first colloidal, thermolabile, and of the nature of an enzyme; the second crystalloidal, dialyzable, thermostable, volatile, and nonvolatile.

Salts of organic acids were present in old culture solutions and organic acids were proved to be highly toxic to tomato plants.

Plants of a resistant strain of tomato were found to be less subject to injury from these toxic materials than plants of a susceptible variety.

These toxic substances produced by *Fusarium lycopersici* also caused wilting of cowpea, soybean, and cabbage. The toxic substances produced by *F. oxysporum* caused wilting of tomato plants.

LITERATURE CITED

(1) Association of Official Agricultural Chemists
1920. official and tentative methods of analysis. as compiled by the committee on revision of methods. revised to nov. 1, 1919. 417 p., illus. Washington, D. C.

(2) Atkinson, G. F.
1892. some diseases of cotton. Ala. Agr. Expt. Sta. Bul. 41, 65 p., illus.

(3) Balls, W. L.
1908. temperature and growth. Ann. Bot. [London] 22: 557–591, illus.

(4) Barnum, C. C.
1924. the production of substances toxic to plants by penicillium expansum link. Phytopathology 14: 238–243, illus.

(5) Brandes, E. W.
1919. banana wilt. Phytopathology 9: 339–389, illus.

(6) Brannon, J. M.
1923. influence of glucose and fructose on growth of fungi. Bot. Gaz. 76: 257–273.

(7) Brooks, C., and Cooley, J. S.
1917. temperature relations of apple-rot fungi. Jour. Agr. Research 8: 139–164, illus.

(8) Brown, W.
1923. experiments on the growth of fungi on culture media. Ann. Bot. [London] 37: 105–129, illus.

(9) Carpenter, C. W.
1918. wilt diseases of okra and the verticillium-wilt problem. Jour. Agr. Research 12: 529–546, illus.

(10) Chupp, C.
1923. diseases of field and vegetable crops in the united states in 1922. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul., Sup. 26: 1–163, illus. [Mimeographed.]

(11) Clayton, E. E.
1923. the relation of temperature to the fusarium wilt of the tomato. Amer. Jour. Bot. 10: 71–88, illus.

(12) CLINTON, G. P.
1904. DISEASES OF PLANTS CULTIVATED IN CONNECTICUT. Conn. State Agr. Expt. Sta. Ann. Rpt. (1903) 27: 279–370, illus.
(13) COONS, G. H.
1922. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1921. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Bul., Sup. 22: 255–414, illus. [Mimeographed.]
(14) DIXON, H. H.
1914. TRANSPIRATION AND THE ASCENT OF SAP IN PLANTS. 216 p., illus. London.
(15) EDGERTON, C. W.
1918. A STUDY OF WILT RESISTANCE IN THE SEED-BED. Phytopathology 8: 5–14, illus.
(16) ——— and MORELAND, C. C.
1913. DISEASES OF THE TOMATO IN LOUISIANA. La. Agr. Expt. Sta. Bul. 142, 23 p., illus.
(17) ——— and MORELAND, C. C.
1920. TOMATO WILT. La. Agr. Expt. Sta. Bul. 174, 54 p., illus.
(18) FAHMY, T.
1923. THE PRODUCTION BY FUSARIUM SOLANI OF A TOXIC EXCRETORY SUBSTANCE CAPABLE OF CAUSING WILTING IN PLANTS. Phytopathology 13: 543–550.
(19) FAWCETT, H. S.
1921. THE TEMPERATURE RELATIONS OF GROWTH IN CERTAIN PARASITIC FUNGI. Calif. Univ. Pubs., Agr. Sci. 4: 183–232, illus.
(20) ———
1924. INFLUENCE OF TIME AND TEMPERATURE ON THE RATE OF GROWTH OF CERTAIN FUNGI. (Abstract) Phytopathology 14: 119–120.
(21) ———
1925. MAINTAINED GROWTH RATES IN FUNGOUS CULTURES OF LONG DURATION. Ann. Appl. Biol. 12: 191–198, illus.
(22) FULTON, H. R.
1907. COTTON WILT. La. Agr. Expt. Sta. Bul. 96, 15 p., illus.
(23) GILBERT, W. W.
1921. COTTON DISEASES AND THEIR CONTROL. U. S. Dept. Agr. Farmers' Bul. 1187, 32 p., illus.
(24) GOSS, R. W.
1921. TEMPERATURE AND HUMIDITY STUDIES OF SOME FUSARIA ROTS OF THE IRISH POTATO. Jour. Agr. Research 22: 65–80, illus.
(25) HARTER, L. L., and WEIMER, J. L.
1923. SOME PHYSIOLOGICAL VARIATIONS IN STRAINS OF RHIZOPUS NIGRICANS. Jour. Agr. Research 26: 363–371.
(26) HASKELL, R. J.
1919. FUSARIUM WILT OF POTATO IN THE HUDSON RIVER VALLEY, NEW YORK. Phytopathology 9: 223–260, illus.
(27) HOPKINS, E. F.
1921. NOTE ON THE HYDROGEN-ION CONCENTRATION OF POTATO DEXTROSE AGAR AND A TITRATION CURVE OF THIS MEDIUM WITH LACTIC ACID. Phytopathology 11: 491–494, illus.
(28) ———
1922. HYDROGEN-ION CONCENTRATION IN ITS RELATION TO WHEAT SCAB. Amer. Jour. Bot. 9: 159–179, illus.
(29) HUMBERT, J. G.
1918. TOMATO DISEASES IN OHIO. Ohio Agr. Expt. Sta. Bul. 321: 159–196, illus.
(30) HURSH, C. R.
1922. THE RELATION OF TEMPERATURE AND HYDROGEN-ION CONCENTRATION TO UREDINIOSPORE GERMINATION OF BIOLOGIC FORMS OF STEM RUST OF WHEAT. Phytopathology 12: 353–361, illus.
(31) LA RUE, C. D., and BARTLETT, H. H.
1922. A DEMONSTRATION OF NUMEROUS DISTINCT STRAINS WITHIN THE NOMINAL SPECIES PESTALOZZIA GUEPINI DESM. Amer. Jour. Bot. 9: 79–92.
(32) LATHROP, E. C.
1917. THE GENERATION OF ALDEHYDES BY FUSARIUM CUBENSE. Phytopathology 7: 14–16.

(33) LEACH, J. G.
1923. THE PARASITISM OF COLLETOTRICHUM LINDEMUTHIANUM. Minn. Agr. Expt. Sta. Tech. Bul. 14, 41 p., illus.
(34) LINK, G. K. K.
1916. A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO. Bot. Gaz. 62: 169–209, illus.
(35) MCCLINTOCK, J. A.
1920. TOMATO WILT. Ga. Agr. Expt. Sta. Bul. 138: 39–49, illus.
(36) MCKENNEY, R. E. B.
1905. I. THE WILT DISEASE OF TOBACCO AND ITS CONTROL. U. S. Dept. Agr., Bur. Plant Indus. Bul. 51: 5–8.
(37) MATSUMOTO, T.
1921. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. XII. PHYSIOLOGICAL SPECIALIZATION IN RHIZOCTONIA SOLANI KÜHN. Ann. Missouri Bot. Gard. 8: 1–62, illus.
(38) ———
1923. FURTHER STUDIES ON PHYSIOLOGY OF RHIZOCTONIA SOLANI KÜHN. Bul. Imp. Col. Agr. and Forestry, Japan 5, 64 p., illus.
(39) MEDALIA, L. S.
1920. "COLOR STANDARDS" FOR THE COLORIMETRIC MEASUREMENT OF H-ION CONCENTRATION P_H 1.2 TO P_H 9.8. Jour. Bact. 5: 441–468, illus.
(40) MELHUS, I. E., MUNCIE, J. H., and HO, W. T. H.
1924. MEASURING WATER FLOW INTERFERENCE IN CERTAIN GALL AND VASCULAR DISEASES. Phytopathology 14: 580–584, illus.
(41) ORTON, W. A.
1900. THE WILT DISEASE OF COTTON AND ITS CONTROL. U. S. Dept. Agr., Div. Veg. Path. Bul. 27, 16 p., illus.
(42) ———
1902. I. THE WILT DISEASE OF THE COWPEA AND ITS CONTROL. U. S. Dept. Agr., Bur. Plant Indus. Bul. 17: 9–20, illus.
(43) OVERTON, J. B.
1911. STUDIES ON THE RELATION OF THE LIVING CELLS TO TRANSPIRATION AND SAP-FLOW IN CYPERUS. I-II. Bot. Gaz. 51: 28–63, 102–120, illus.
(44) PICADO, C.
1923. SUR L'ACTION À DISTANCE DES CHAMPIGNONS PHYTOPATHOGÈNES. Cong. Path. Veg. Strasbourg, 1923, p. 28–34. Strasbourg.
(45) POOLE, R. F.
1922. RECENT INVESTIGATIONS ON THE CONTROL OF THREE IMPORTANT FIELD DISEASES OF SWEET POTATOES. N. J. Agr. Expt. Sta. Bul. 365, 39 p., illus.
(46) ———
1922. SWEET POTATO DISEASES IN NEW JERSEY. N. J. Agr. Expt. Sta. Circ. 141, 31 p., illus.
(47) PRATT, C. A.
1924. THE STALING OF FUNGAL CULTURES. I. GENERAL AND CHEMICAL INVESTIGATION OF STALING BY FUSARIUM. Ann. Bot. [London] 38: 563–595, illus.
(48) ———
1924. THE STALING OF FUNGAL CULTURES. II. THE ALKALINE METABOLIC PRODUCTS AND THEIR EFFECT ON THE GROWTH OF FUNGAL SPORES. Ann. Bot. [London] 38: 599–615, illus.
(49) SCOTT, I. T.
1924. THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF FUSARIUM LYCOPERSICI AND ON TOMATO WILT. Missouri Agr. Expt. Sta. Research Bul. 64, 32 p., illus.
(50) SHAPOVALOV, M., and LESLEY, J. W.
1924. THE BEHAVIOR OF CERTAIN VARIETIES OF TOMATOES TOWARDS FUSARIUM-WILT INFECTION IN CALIFORNIA. Phytopathology 14: 188–197, illus.
(51) SHERBAKOFF, C. D.
1915. FUSARIA OF POTATOES. N. Y. Cornell Agr. Expt. Sta. Mem. 6: 97–270, illus.

(52) SHERWOOD, E. C.
1923. HYDROGEN-ION CONCENTRATION AS RELATED TO THE FUSARIUM WILT OF TOMATO SEEDLINGS. Amer. Jour. Bot. 10: 537–553, illus.
(53) SIDERIS, C. P.
1925. STUDIES ON THE BEHAVIOR OF FUSARIUM CROMYOPHTHORON IN CARBOHYDRATES, GLUCOSIDES, PROTEINS AND VARIOUS DECOCTIONS, WITH A DISCUSSION ON THE "ISOMETABOLIC POINT" OF SUBSTANCES. Phytopathology 15: 129–145.
(54) SMITH, E. F.
1899. WILT DISEASE OF COTTON, WATERMELON, AND COWPEA (NEOCOSMOSPORA NOV. GEN.). U. S. Dept. Agr., Div. Veg. Path. Bul. 17, 72 p., illus.
(55) STEVENS, F. L.
1898. THE EFFECT OF AQUEOUS SOLUTIONS UPON THE GERMINATION OF FUNGUS SPORES. Bot. Gaz. 26: 377–406, illus.
(56) TARR, L. W., and NOBLE, S. C.
1922. THE EFFECT OF HYDROGEN-ION CONCENTRATION UPON THE GROWTH OF SEEDLINGS. Del. Agr. Expt. Sta. Bul. 131, 52 p., illus. (Tech. Bul. 1.)
(57) TISDALE, W. H.
1917. FLAXWILT: A STUDY OF THE NATURE AND INHERITANCE OF WILT RESISTANCE. Jour. Agr. Research 11: 573–606, illus.
(58) UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY.
1923. FUSARIUM WILT OF TOMATO COMMON THIS YEAR. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. 7: 73. [Mimeographed.]
(59) WEBB, R. W.
1919. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. X. GERMINATION OF THE SPORES OF CERTAIN FUNGI IN RELATION TO HYDROGEN-ION CONCENTRATION. Ann. Missouri Bot. Gard. 6: 201–222, illus.
(60) WHITE, R. P.
1926. TOMATO WILT INVESTIGATIONS. Kans. Agr. Expt. Sta. Tech. Bul. 20, 32 p., illus.

A COMPARISON OF SOME PHYSICAL AND CHEMICAL TESTS FOR DETERMINING THE QUALITY OF GLUTEN IN WHEAT AND FLOUR[1]

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INTRODUCTION

When flour is made into dough and fermentation is allowed to take place the gluten stretches and forms eventually, when the dough is baked, the supporting structure of the loaf of bread. The character of the resulting loaf, or, in other words, the baking value of the flour, is determined in part by the peculiar properties of the principal flour proteins, glutenin and gliadin, which are capable, in the presence of water, of forming a tough, coherent, and elastic mass.

Inasmuch as these gluten proteins constitute a fairly definite and uniform portion of the total protein in wheat flour, the Kjeldahl method (*2, p. 5–6*)[3] or its modification has proved a fairly satisfactory and dependable index of the quantity of gluten present.

In spite of the immense amount of research devoted to the subject, not as much as should be is known about the gluten quality of flour, although notable contributions have been made to the subject recently.

Outstanding among the tests used by different laboratories in obtaining indices of gluten quality are those for kernel texture, the washed-gluten, the water-absorbing power of the flour, the percentage of crude protein in the wheat or flour, and of late the viscometric procedure of Sharp and Gortner (*17*). Many short cuts and modifications of the last-named test have been tried, with a view to applying it as a routine method for estimating gluten quality in wheat or wheat flours.

With the exception of a recent paper by Blish and Sandstedt (*4*), very few data have been found that compare methods for rating gluten quality with the observed baking data on the same flour. The subject matter of this paper, therefore, concerns itself with a study of the results obtained by several methods now in use for determining gluten quality and compares these data with the baking data on the same samples of flour. The baking methods used are described in United States Department of Agriculture Bulletin No. 1187 (*21*). All bakings were made on straight grade flours.

For ease in comparing the value of each physical or chemical test as an indicator of gluten quality, the data obtained from a study of each method have been worked up on a statistical basis, by the Pearson method (*15, p. 373*). Such correlations are of practical value, as they place the cereal chemist in a better position to judge

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[2] The milling and baking laboratory, Bureau of Agricultural Economics, U. S. Department of Agriculture, J. H. Shollenberger in charge, supplied the baking data on the samples discussed in this article.

[3] Reference is made by number (italic) to "Literature cited," p. 263.

the value of certain determinations in their application to the milling and baking industry.

Samples of wheat and flour representing the two crop years 1923 and 1924 were studied. The samples of the 1923 wheat crop were commercial samples that had been graded at the various Federal grain supervision offices located in the hard red spring and hard red winter wheat areas. The great majority of the 1924 wheat crop samples were grown by the Office of Cereal Investigations, Bureau of Plant Industry. They represent pure varieties of well-known hard red spring and hard red winter wheats.

In making these studies each wheat and each flour sample had all of these tests applied to it in so far as time and material permitted. Other accessory data were accumulated as the investigation progressed and are introduced from time to time to support the discussion.

Tests were made comparing the texture (or percentage of dark, hard, and vitreous kernels in the wheat) with the volume of the loaf baked from flour milled from the same lot of wheat; the protein[4] content of the wheat and of the flour milled from the wheat and the associated baking tests; the percentage of wet and dry gluten in the various flours and the volume of the loaf baked from these flours; the water-absorbing power of the flours and the volume of the loaf baked from these flours; and the viscosity as measured by the viscometric methods of Sharp and Gortner (*17*), with and without modifications; and the volume of the baked loaf of bread.

TEXTURE OF KERNEL AS AN INDEX OF BAKING QUALITY

It has long been known that there is a relationship between dark and vitreous kernels of wheat and their protein content. As has been reported earlier by the authors (*5*) and recently by Shollenberger and Coleman (*20*), this relationship is particularly noticeable if the dark, spotted, or starchy kernels are removed from the same sample of wheat and analyzed.

Frank (*8*) and Mangels and Sanderson (*13*) have reported figures comparing the protein content of different lots of wheat and the percentage of dark and vitreous kernels in these lots, and their conclusions are that high protein content is not always associated with a high percentage of dark, hard, and vitreous kernels.

Correlation studies made in the grain research laboratory, Bureau of Agricultural Economics, comparing the protein content and the percentage of dark, hard, and vitreous kernels in 500 samples of hard red spring and hard red winter wheat grown in the crop years 1923 and 1924, gave the coefficients shown in Table 1.

[4] The usual procedure of multiplying the percentage of nitrogen by the factor 5.7 and expressing the result as protein has been adopted. It should be pointed out that approximately 25 per cent of such protein is nongluten in nature, and the protein figures listed really represent crude protein.

TABLE 1.—*Correlation between protein content of wheat and the percentage of dark, hard, and vitreous kernels of hard red spring and hard red winter wheats, crop of 1923 and 1924*

Number of samples	Percentage of protein			Percentage of dark, hard, and vitreous kernels			Coefficient of correlation	Probable error	Class of wheat	Crop year
	Average	Maximum	Minimum	Average	Maximum	Minimum				
128	12.65	15.20	9.20	81.0	100.0	25.2	+0.6414	+0.0351	Hard red spring	1923
113	13.70	18.89	9.50	89.5	99.9	18.0	+.3981	+.0536	do	1924
183	12.00	15.90	8.20	67.5	99.9	6.0	+.6004	+.0319	Hard red winter	1923
76	12.90	18.00	8.40	78.4	100.0	18.2	+.5593	+.0532	do	1924

The coefficient of correlation obtained from 183 samples of hard red winter wheat grown in 1923 was $+0.6004 \pm 0.0319$, whereas that obtained from 76 samples of the 1924 crop was $+0.5593 \pm 0.0532$. From a study of 128 samples of the 1923 hard red spring wheat crop a coefficient of $+0.6414 \pm 0.0351$ was obtained, as compared with $+0.3981 \pm 0.0536$, obtained from 113 samples of hard red spring wheat of the 1924 crop.

In each of the above cases the relationship was such that it was apparent that an estimation of the percentage of dark, hard, and vitreous kernels was only a general index of the protein content of the wheat. A low percentage of dark kernels did usually indicate a low protein content, and sometimes a high percentage of dark and hard kernels was associated with high protein content, but as an exact measure of the protein content of wheat this determination is not reliable.

But inasmuch as it is now recognized that quality of gluten, as well as quantity, is a factor influencing baking strength and that high quality is usually associated with the hard and vitreous kernels, it was decided to compare the texture of the wheat samples with the baking data obtained with the same samples. Tables 2 and 3 give the distribution of the data, as well as the correlation coefficients obtained from this comparison. Complete data are given for the bakings made of 183 samples of the hard red winter 1923 wheat crop and 113 samples of the hard red spring 1924 wheat crop.

TABLE 2.—*Correlation between kernel texture and loaf volume of hard red winter wheat, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of dark, hard, and vitreous kernels										
	5–14.9	15–24.9	25–34.9	35–44.9	45–54.9	55–64.9	65–74.9	75–84.9	85–94.9	95 and over	Total
1,700 to 1,799						1					1
1,800 to 1,899						1		1	1	3	6
1,900 to 1,999	2			2	2	3	4	4	1	8	26
2,000 to 2,099			3	7	6	7	12	5	1	6	47
2,100 to 2,199		1	3	7	3	8	17	4	3	4	50
2,200 to 2,299	1		3	4	0	5	5	3	6	2	35
2,300 to 2,399							1	2	2	2	7
2,400 to 2,499								1	7	0	8
2,500 to 2,599									1	1	2
2,600 to 2,699										1	1
Total	3	1	9	20	17	25	39	20	22	27	183

$r = +0.0990 \pm 0.0494$.

TABLE 3.—*Correlation between kernel texture of wheat and loaf volume of hard red spring wheat, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of dark, hard, and vitreous kernels										
	0–9.9	10–19.9	20–29.9	30–39.9	40–49.9	50–59.9	60–69.9	70–79.9	80–89.9	90–100.0	Total
1,900 to 1,999								1	3	5	9
2,000 to 2,099		1			1			1	6	3	12
2,100 to 2,199						1	1	3	7	19	31
2,200 to 2,299								3	3	12	18
2,300 to 2,399					1	1		4		12	18
2,400 to 2,499								1		5	6
2,500 to 2,599									1	5	6
2,600 to 2,699									1	4	5
2,700 to 2,799										4	4
2,800 to 2,899							1		1		2
2,900 to 2,999										1	1
3,000 to 3,099										1	1
Total		1			2	2	2	13	22	71	113
$r = +0.1517 \pm 0.0620$.											

The distribution tables listing the data for the 128 samples of the 1923 hard red spring wheat crop and for the 76 samples of the 1924 hard red winter wheat crop are not shown, as they do not add to the information. Suffice it to say that the correlation coefficients were $+0.2612 \pm 0.0556$ for the 128 samples of hard red spring wheat and $+0.0287 \pm 0.0773$ for the 76 samples of hard red winter wheat.

The coefficients obtained from the 183 samples of the 1923 hard red winter wheat crop and from the 113 samples of the 1924 hard red spring wheat crop—namely $+0.0990 \pm 0.0494$ and $+0.1517 \pm 0.0620$, respectively—are of only minor significance. It is evident, therefore, that texture of kernel is of questionable value as an index of baking strength.

In making the above conparisons only the loaf volume of the bread has been considered as an index of gluten quality. It is well known, however, that loaf volume can be materially increased at the expense of the texture of the crumb. An attempt was made to offset this contingency by taking 100 points as a perfect crumb-texture score and by making, for every 1 per cent drop in the quality of crumb texture a 1 per cent deduction from the observed volume of the baked loaf. That is to say, the loaf volume in cubic centimeters was multiplied by the crumb-texture score of the loaf, and the resulting figure was compared with the kernel texture of the wheat in the same manner in which the original loaf volume was compared with the precentage of virteous kernels. When this method of assigning baking values was used, the coefficients reported above for the 1923 and 1924 hard red winter wheat crop changed from $+0.0990 \pm 0.0494$ and $+0.0287 \pm 0.0773$ to $+0.1027 \pm 0.0493$ and $+0.0328 \pm 0.0773$ respectively.

For the samples of the 1923 and 1924 hard red spring wheat crops the coefficients changed from $+0.2612 \pm 0.0556$ and $+0.1517 \pm 0.0620$ to $+0.2937 \pm 0.0545$ and $+0.1249 \pm 0.0625$. This method of valuation did not materially change the coefficients; thus, no change in the earlier deductions can be made.

PROTEIN CONTENT OF WHEAT AND LOAF VOLUME OF BREAD

During the last few years the protein content of wheat has been assuming increased importance in determining the market price of wheat. Transactions are known where as much as 30 to 40 cents a bushel premium were paid for wheat of the same numerical grade but of higher protein content.

Such investigations as those of Zinn (*26*), Bailey (*3*), Thomas (*24*), Stockham (*23*), Sherwood (*18, 19*), and Mangels (*12*), wherein the protein content of the wheat has been compared with the baking data, have given evidence which tends to substantiate the practice and value of judging baking strength by a determination of the protein content of the wheat. It is agreed that there are individual cases in which the relationship between the percentage of protein in the wheat and the associated baking test does not hold because of the influence of other factors that affect baking strength; yet, when purchases are made on a large scale and the wheat is blended, the individual differences seem to disappear, and a close relationship is shown to exist.

In Tables 4 and 5 are given the distribution data and the correlation coefficients obtained by comparing the protein content of 183 samples of hard red winter wheat grown in 1923 and of 116 samples of hard red spring wheat grown in 1924 with the associated loaf volumes.

TABLE 4.—*Correlation between protein content of wheat and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in wheat									
	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	Total
1,700 to 1,799	1									1
1,800 to 1,899			2	1	3					6
1,900 to 1,999	1	5	2	6	4	7	1			26
2,000 to 2,099	4	1	5	23	10	2	2			47
2,100 to 2,199		1	5	23	12	7		2		50
2,200 to 2,299			5	13	9	8				35
2,300 to 2,399					2	4	1			7
2,400 to 2,499					1	4	1	1	1	8
2,500 to 2,599							1	1		2
2,600 to 2,699							1			1
Total	6	7	19	66	41	32	7	4	1	183
$r=+0.4281 \pm 0.0407$										

TABLE 5.—*Correlation between protein of wheat and loaf volume of hard red spring wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in wheat										
	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	17–17.99	18–9 18.9	Total
1,900 to 1,999	1	2	2		2	1	1		1		10
2,000 to 2,099		4	3	3	1	2	1				14
2,100 to 2,199	1	4	5	3	4	7	4		2		30
2,200 to 2,299			1	7	4		5	1	1		19
2,300 to 2,399			1	3	3	4	3	3	1		18
2,400 to 2,499				1	1	2	2				6
2,500 to 2,599			1		2	1	1	1			6
2,600 to 2,699					2	1	1	1			5
2,700 to 2,799					1	1		1	1		4
2,800 to 2,899						1	1				2
2,900 to 2,999										1	1
3,000 to 3,099							1				1
Total	2	10	13	17	20	20	20	7	6	1	116
$r=+0.4184 \pm 0.0517$											

A study of Tables 4 and 5 will show that a positive, somewhat significant relationship exists between the protein content of the wheat and the volume of loaf made from it. With the samples of the 1923 hard red winter wheat crop the coefficient between the protein content and loaf volume was $+0.4281 \pm 0.0407$, and with those of the 1924 hard red spring crop it was $+0.4184 \pm 0.0517$.

The coefficient of correlation between the protein content of the 1924 hard red winter wheat crop and the associated loaf volume was -0.0370 ± 0.0773.

An examination of Table 6 shows that there was a decided relation between the loaf volume of the bread and the protein content of the wheat up to and including the 12 per cent protein area. When the 13 per cent area was reached, however, there was a decided break in the quality of the gluten, as 20 of the 34 samples of wheat having 13 per cent of protein and over produced loaves of bread having a volume of 1,999 cubic centimeters or less. From a statistical angle, therefore, these samples counterbalanced the very high relationship which would have been found with the samples of wheat that had a protein content of 8 to 12 per cent.

A coefficient of $+0.4610 \pm 0.0407$ was obtained when the baking data were compared with the protein content of the 128 samples of spring wheat flour from the crop of 1924.

When the loaf-volume figures were corrected for variations in the texture of the crumb, the coefficients were slightly smaller. In the case of the 183 samples of the 1923 hard red winter wheat crop the coefficient changed from $+0.4281 \pm 0.0407$ to $+0.3932 \pm 0.0422$. For the 116 samples of the 1924 hard red spring wheat crop the coefficient changed from $+0.4184 \pm 0.0517$ to $+0.3694 \pm 0.0546$. The coefficient -0.0370 ± 0.0773 for the 76 samples of hard red winter wheat of the crop of 1924 changed to -0.0353 ± 0.0773, and the coefficient $+0.4610 \pm 0.0470$, obtained from the 128 samples of hard red spring wheat, crop of 1923, changed to $+0.4068 \pm 0.0498$, when texture of crumb was considered.

TABLE 6.—*Correlation between protein content of wheat and loaf volume of hard red winter wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in wheat											
	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	17–17.99	18–18.99	Total
1,600 to 1,699	2											2
1,700 to 1,799	1							1				2
1,800 to 1,899	3	1						4	1	4		13
1,900 to 1,999		5		1	1	1	1	1	5	2		17
2,000 to 2,099		5	1		2	1	1	2			1	13
2,100 to 2,199		1	2	2	4	4			2			15
2,200 to 2,299			1	5	1				1			8
2,300 to 2,399				1	1							2
2,400 to 2,499								1				1
2,500 to 2,599					2	1						3
Total	6	12	4	9	11	7	2	9	9	6	1	76

$r = -0.0370 \pm 0.0773$.

PROTEIN CONTENT OF FLOUR AND LOAF VOLUME OF BREAD

Inasmuch as only 75 to 80 per cent of the crude protein content of wheat is really gluten protein, studies were next made comparing the protein content of straight grade flours with the associated loaf volumes just discussed. These data are given in full for the samples of the 1923 hard red winter and 1924 hard red spring wheat crops. The distribution data and coefficients of correlation are shown in Tables 7 and 8.

Although it would seem reasonable to expect a higher coefficient of correlation by plotting protein content of flour against loaf volume, slightly lower coefficients were obtained in each case, namely, $+0.4249 \pm 0.0409$ for the 183 flour samples of the 1923 crop of hard red winter wheat and $+0.4005 \pm 0.0526$ for the 116 samples of the 1924 hard red spring wheat. The coefficient obtained for 76 samples of the hard red winter wheat crop of 1924 was $+0.1153 \pm 0.0763$, whereas a coefficient of $+0.4641 \pm 0.0468$ was obtained for the 128 samples of the hard red spring wheat crop of 1923.

TABLE 7.—*Correlation between protein content of flour and loaf volume of hard red winter wheat, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in flour									
	7–7.99	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	05–15.99	Total
1,700 to 1,799	1									1
1,800 to 1,899			2	1	3					6
1,900 to 1,999	2	2	5	6	9	7	1			26
2,000 to 2,099	4	3	9	19	7	3	2			47
2,100 to 2,199		2	9	19	11	5	3	1		50
2,200 to 2,299			7	13	9	5	1			35
2,300 to 2,399					1	5	1			7
2,400 to 2,499				1		1	4	1	1	8
2,500 to 2,599							1	1		2
2,600 to 2,699							1			1
Total	7	7	32	59	34	26	14	3	1	183
$r = +0.4249 \pm 0.0409$.										

TABLE 8.—*Correlation between protein content of flour and loaf volume of hard red spring wheat, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of protein in flour										
	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	17–17.99	Total
1,900 to 1,999	1	1	1	2	1	2					8
2,000 to 2,099		5	2	2	2	1	3				15
2,100 to 2,199			7	7	1	5	6	4	1		31
2,200 to 2,299			1	9	1	2	3	2	1		19
2,300 to 2,399			1	4	2	3	4	4		1	19
2,400 to 2,499				1		2	3				6
2,500 to 2,599		1			1	1	1	1	1		6
2,600 to 2,699						2	1	1			4
2,700 to 2,799						1	1	2			4
2,800 to 2,899				1			1				2
2,900 to 2,999									1		1
3,000 to 3,099							1				1
Total	1	7	12	26	8	19	24	14	4	1	116
$r = +0.4005 \pm 0.0526$.											

When the differences in texture of the crumb were taken into consideration, lower coefficients were obtained in every case. The coefficient for the 183 samples of the hard red winter wheat crop of 1923 changed from $+0.4249 \pm 0.0409$ to $+0.3816 \pm 0.0426$; that for the 116 samples of 1924 hard red spring wheat, from $+0.4005 \pm 0.0526$ to $+0.3278 \pm 0.0559$; that for the 76 samples of 1924 hard red winter wheat, from $+0.1153 \pm 0.0763$ to $+0.0464 \pm 0.0772$; and that for the 128 samples of the 1923 crop of hard red spring wheat, from $+0.4641 \pm 0.0468$ to $+0.3856 \pm 0.0508$.

It appears from the above data, therefore, that under the conditions under which these samples were baked, nothing is gained by making a protein determination on the flour rather than on the wheat. Samples of very low protein content were generally associated with low loaf volume. On the other hand, wheats containing over 13 per cent of protein appeared to contain a weaker quality of protein or gluten than those containing 11 or 12 per cent.

WET AND DRY GLUTEN CONTENT OF FLOUR AND LOAF VOLUME

The wet and dry gluten determination is still used by some chemists. It is argued by the supporters of this test that, in addition to the quantitative feature of the test, a great deal can be learned regarding the quality of the protein or gluten in the wheat or flour sample.

For the purpose of comparing the value of this test with that of the other tests described in this paper dry-gluten determinations were made on 118 samples of hard red winter flour and on 82 samples of hard red spring wheat flour. All the samples were from the 1923 crop.

The data comparing the dry-gluten determinations with the associated loaf volumes are shown in Tables 9 and 10. With the data from the 82 samples of spring wheat flour a coefficient of $+0.2271 \pm 0.0706$ was obtained, whereas the coefficient determined from the data from the 118 samples of the hard red winter flour was $+0.4151 \pm 0.0514$. When the loaf-volume figures were corrected for differences in texture of the crumb, the coefficients changed from $+0.2271 \pm 0.0706$ to $+0.0833 \pm 0.0740$ for the hard red spring wheat flour and from $+0.4151 \pm 0.0514$ to $+0.4181 \pm 0.0512$ for the hard red winter wheat flour.

It would appear from a study of the distribution data and the coefficient of correlation that this determination is of greater value than the kernel-texture determination and almost as good as the crude-protein determination as an indicator of baking quality. The great drawback to this determination, of course, is the difficulty that other laboratories and chemists have in duplicating it. Undeniably differences in the composition of the wash water, the care with which the starch and other adhering material is removed from the dough ball, and the differences in the composition of the flour, which occur when different laboratories extract the flour from the same wheat for the gluten tests, exert far-reaching influences on the results obtained.

As a matter of routine carried on by a single individual at a particular place, the test probably does have its uses, because the operator has the advantage of observing something of the nature of the gluten in the flour sample.

TABLE 9.—*Correlation between percentage of dry gluten and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of dry gluten										
	8–8.99	9–9.99	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	16–16.99	17–17.99	Total
1,900 to 1,999	1		1	4	2	3					11
2,000 to 2,099		3	10	10	3	2	1				29
2,100 to 2,199		1	15	7	5	3	1	1			33
2,200 to 2,299		2	8	9	4	3	1				27
2,300 to 2,399				2	1	2		1			6
2,400 to 2,499				2		3	1	2		1	9
2,500 to 2,599							1	1			2
2,600 to 2,699						1					1
Total	1	6	34	34	15	17	5	5		1	118
$r = +0.4151 \pm 0.0514$.											

TABLE 10.—*Correlation between percentage of dry gluten and loaf volume of hard red spring wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of dry gluten						
	10–10.99	11–11.99	12–12.99	13–13.99	14–14.99	15–15.99	Total
1,800 to 1,899		1					1
1,900 to 1,999				1			1
2,000 to 2,099	1	2	1	3	1		8
2,100 to 2,199		3	15	11	4	1	34
2,200 to 2,299	1	1	8	5	2		17
2,300 to 2,399		3	4	1	3	2	13
2,400 to 2,499		1		2	4		7
2,500 to 2,599				1			1
Total	2	11	28	24	14	3	82
$r = +0.2271 \pm 0.0705$.							

The wet-gluten determination is also sometimes associated with baking quality. It has not come into very general use because of the extreme difficulty of making the test correctly and consistently. Nevertheless, correlation studies were made from the data obtained from 119 samples of flour from the 1923 crop of hard red winter wheat and from 106 samples of the flour milled from the 1923 crop of hard red spring wheat. The results are given in Tables 11 and 12. It will be seen from the data presented that the coefficient obtained from a study of the 119 samples of hard red winter wheat flour was $+0.4806 \pm 0.0475$. For the 106 samples of hard red spring wheat flour a small coefficient was obtained, $+0.1355 \pm 0.0643$.

TABLE 11.—*Correlation between percentage of wet gluten and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of wet gluten										
	22–24.99	25–27.99	28–30.99	31–33.99	34–36.99	37–39.99	40–42.99	43–45.99	46–48.99	49–51.99	Total
1,800 to 1,899				1							1
1,900 to 1,999	1		2	1	1	2	1				8
2,000 to 2,099	1	5	8	8	1	3	1				27
2,100 to 2,199		2	12	14	4	3	2		1		38
2,200 to 2,299		3	9	5	8	2	2	1			30
2,300 to 2,399					2	1		1			4
2,400 to 2,499				1			3	1	2	1	8
2,500 to 2,599							1		1		2
2,600 to 2,699							1				1
Total	2	10	31	30	16	11	11	3	4	1	119
$r=+0.4806\pm0.0475$.											

TABLE 12.—*Correlation between percentage of wet gluten and loaf volume of hard red spring wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of wet gluten									
	25–27.99	28–30.99	31–33.99	34–36.99	37–39.99	40–42.99	43–45.99	46–48.99	49–51.99	Total
1,800 to 1,899			1			1				2
1,900 to 1,999			2	1	1		1			5
2,000 to 2,099	1	2	2	3	2		1	1		12
2,100 to 2,199	1	6	17	13	5	2	3	2		49
2,200 to 2,299		2	6	3	5				2	18
2,300 to 2,399		2	4	1	1	4		1		13
2,400 to 2,499					2	4				6
2,500 to 2,599				1						1
Total	2	12	32	22	16	11	5	4	2	106
$r=+0.1355\pm0.0643$.										

WATER-ABSORBING POWER OF FLOUR AND LOAF VOLUME

The water-absorbing power of flour is important to the baker, as it is usually thought to be a good index of the number of loaves of bread which can be obtained from a given weight of flour.

Correlation studies were therefore made, comparing the water-absorbing power of the flour with the volume of the baked loaf.

Distribution data are given in Tables 13 and 14 for samples of the 1923 hard red winter crop and for samples of the 1924 hard red spring crop. No relationship was found between the water-absorbing power of the flour and the volume of the resulting loaf of bread.

TABLE 13.—*Correlation between water-absorbing power of flour and loaf volume of hard red winter wheat, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of water absorption																	
	53–53.9	54–54.9	55–55.9	56–56.9	57–57.9	58–58.9	59–59.9	60–60.9	61–61.9	62–62.9	63–63.9	64–64.9	65–65.9	66–66.9	67–67.9	68–68.9	69–69.9	Total
1,700 to 1,799								1										1
1,800 to 1,899									1	2	1		1		1			6
1,900 to 1,999	1	1	2		3	2	3	2	2	3				3	3		1	26
2,000 to 2,099		1	1	6	8	6	4	7	2	2	4	4	1			1		47
2,100 to 2,199		2	1	5	6	6	6	6	8	6	1	3						50
2,200 to 2,299		2	2	3	2	2	3	10	6		2	1	2					35
2,300 to 2,399						1	2	1	1	1	1							7
2,400 to 2,499					1			2		2		1	2					8
2,500 to 2,599								1		1								2
2,600 to 2,699									1									1
Total	1	6	6	14	20	17	18	30	21	17	9	9	6	3	4	1	1	183

$r = -0.0358 \pm 0.0498$.

TABLE 14.—*Correlation between water-absorbing power of flour and loaf volume of hard red spring wheat, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of water absorption																	
	52–52.9	53–53.9	54–54.9	55–55.9	56–56.9	57–57.9	58–58.9	59–59.9	60–60.9	61–61.9	62–62.9	63–63.9	64–64.9	65–65.9	66–66.9	67–67.9	Total
1,900 to 1,999				1		1	2	1	1		2			1			9
2,000 to 2,099				2		2	3	2	1		1		3	1	1		16
2,100 to 2,199	1		1		1	1	4	7	5	2	2	2	3	1		1	31
2,200 to 2,299		1	2	1	1	1	1	2	3	1	1	3	1	1			19
2,300 to 2,399			1	3	1	3			2	1	2	2	1	1			17
2,400 to 2,499					1	2	1				1			1			6
2,500 to 2,599				1				1	1		1		1		1		6
2,600 to 2,699						1			1	1	1	1					5
2,700 to 2,799				1				1			1	1					4
2,800 to 2,899					1				1								2
2,900 to 2,999											1						1
3,000 to 3,099												1					1
Total	1	1	4	9	5	11	11	14	15	5	13	10	9	6	2	1	117

$r = +0.0341 \pm 0.0623$.

TABLE 15.—*Correlation between water-holding capacity of flour and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to percentage of water-holding capacity																
	66–66.9	67–67.9	68–68.9	69–69.9	70–70.9	71–71.9	72–72.9	73–73.9	74–74.9	75–75.9	76–76.9	77–77.9	78–78.9	79–79.9	80–80.9	81–81.9	Total
1,700 to 1,799								1									1
1,800 to 1,899										2	2		1		1		6
1,900 to 1,999	1	1		1	1	6	3	1	1	3	1			3	3	1	26
2,000 to 2,099			1	2	7	7	7	5	7	1	5	3		1	1		47
2,100 to 2,199			1	3	3	9	8	5	8	5	6	1	1				50
2,200 to 2,299		1	3	2	2	2	4	8	3	5		3	1	1			35
2,300 to 2,399						1	2		1	2	1						7
2,400 to 2,499					1				2	1	1	1	1	1			8
2,500 to 2,599								1		1							2
2,600 to 2,699									1								1
Total	1	2	5	8	14	25	24	21	23	20	16	8	4	6	5	1	183

$r = -0.0289 \pm 0.0498$.

With the 183 samples hard red winter wheat flour samples of the 1923 crop the coefficient was small and negative, namely, -0.0358 ± 0.0498. For the 117 samples of spring wheat flour milled from the 1924 crop the coefficient was small and positive, $+0.0341 \pm 0.0623$. The 76 flour samples of the 1924 hard red winter crop yielded a negative coefficient of -0.3045 ± 0.0702, whereas from the flour milled from the 128 samples of the 1923 grown spring wheat a negative coefficient of -0.0187 ± 0.0596 was obtained.

On the possibility that a more significant correlation might result if the water within the flour before the water-absorbing test was made was considered, a new set of correlations was computed, which included this moisture. The data obtained by correlating these new values with the baking tests on the same samples are given in Tables 15 and 16.

TABLE 16.—*Correlation between water-holding capacity of flour and loaf volume of hard red spring wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to percentage of water-holding capacity															
	66–66.9	67–67.9	68–68.9	69–69.9	70–70.9	71–71.9	72–72.9	73–73.9	74–74.9	75–75.9	76–76.9	77–77.9	78–78.9	79–79.9	80–80.9	Total
1,900 to 1,999				1	1	1	1	1	1	2				2		10
2,000 to 2,099		1	1	2	2	3	1	2	1		1	1	2		1	18
2,100 to 2,199	1		1		1	2	5	6	5	2	1	4	1		1	30
2,200 to 2,299		2		1		3	3	2	2	3	3	1	1			21
2,300 to 2,399		1	2	2		3		1	4	2	1	2	1			19
2,400 to 2,499					2	1						1		1		5
2,500 to 2,599							1					1		1		3
2,600 to 2,699									1	2						3
2,700 to 2,799				1			1		1		1					4
2,800 to 2,899									1							1
2,900 to 2,999				1							1					2
3,000 to 3,099												1				1
Total	1	4	4	8	6	13	12	12	16	11	8	11	5	4	2	117

$r = +0.0556 \pm 0.0622$.

The resulting coefficients are such that no confidence can be placed in this test as an index of gluten quality or of baking strength.

When a comparison is made between the coefficients obtained when the water in the flour was taken into consideration and those obtained when it was not taken into consideration, it is found that the coefficient obtained from the 183 samples of flour of the 1923 winter wheat crop changed from -0.0358 ± 0.0498 to -0.0289 ± 0.0498; that from the 117 samples of flour milled from the 1924 hard red spring wheat crop changed from $+0.0341 \pm 0.0623$ to $+0.0556 \pm 0.0622$; that from the 76 samples of the 1924 hard red winter wheat crop changed from -0.3045 ± 0.0702 to -0.3134 ± 0.0698; and that from the 128 samples of the 1923 hard red spring wheat crop changed from -0.0187 ± 0.0596 to -0.0164 ± 0.0596.

VISCOSITY OF FLOUR IN WATER SUSPENSIONS AS AN INDEX OF GLUTEN QUALITY

Stimulated by the earlier researches of Weaver and Goldtrap (*25*) and as a direct result of the investigations of Gortner and Sharp (*17*), several viscometric methods for measuring the gluten quality have come into prominence. In the Gortner and Sharp method (*17*), gluten quality is measured by determining the slope of the curve formed by plotting the logarithm of the viscosities as ordinates against

the logarithm of the flour concentration as abscissas. When this is done, it is found that the values lie on a straight line. The increment of increase of the logarithm of the viscosity per increment of increase of the logarithm of flour concentration determines the slope of this line. The equation is log. viscosity $= a - b$ (log. concentration) where a and b are constants. The position of the line on the viscosity axis at zero flour concentration is determined by a, and b is the tangent of the angle which the line makes with the horizontal. Thus b is directly related to the slope of the line and may be taken as a numerical value of gluten quality. The value b may be measured from the plotted line or computed by the method of least squares (*14*).

As the method prescribed by Gortner and Sharp (*17*) was considered too long and involved for rapid laboratory or control work, numerous short cuts and modifications of it have been tried, with varying success. Investigations of this kind have been reported from time to time in such papers as those of Morgan (*16*), Blish and Sandstedt (*4*), Smith (*22*), and Durham (*6*).

In all these investigations a determination of the viscosity of one concentration of flour or wheat, as the case might be, received the most attention. Some investigators washed the flour or wheat free from electrolytes, whereas others ran their tests on the sample without washing it.

Fundamentally, the one-concentration idea is unsound, as has been pointed out by Gortner (*9*), but for that matter the whole principle of measuring a plastic material like flour with a torsional viscosimeter, such as the MacMichael (*10, p. 349*), is subject to criticism.

Nevertheless, preliminary studies were made of the same samples of the 1923 hard red winter and hard red spring wheat flour that had been studied before. The one-cencentration plan was used, namely, washing 20 gm. of flour free from electrolytes with 2,000 c. c. of distilled water and then making it to a 100 c. c. volume.

The MacMichael viscosimeter (*11*) was used, in which were employed a No. 30 wire, a 2 c. c. bob, and a cup making 76 turns per minute. Suspensions were poured into the cup of the viscosimeter until the 2 cm. mark on the bob was reached.

Normal lactic acid was added to each flour-and-water suspension until each flour was the same in respect to hydrogen-ion concentration.

The data from this study are given in Tables 17 and 18.

TABLE 17.—*Correlation between viscosity of one concentration of flour and water and loaf volume of hard red winter wheat flours, crop of 1923*

Loaf volume, in cubic centimeters	Number of samples according to viscosity in degrees MacMichael															
	100–124	125–149	150–174	175–199	200–224	225–249	250–274	275–299	300–324	325–349	350–374	375–399	400–424	425–449	450–474	475–499	Total
1,700 to 1,799		1															1
1,800 to 1,899					2	3	1										6
1,900 to 1,999	1	1	4	5	2	5	3	2			2					1	26
2,000 to 2,099	1	4	6	12	11	4	3	1	1		2	1	1				47
2,100 to 2,199			5	6	18	12	3	1	3	1	1						50
2,200 to 2,299	1	2		9	6	8	7	2									35
2,300 to 2,399					1	3	2	1									7
2,400 to 2,499					1	1		2	4								8
2,500 to 2,599									1	1							2
2,600 to 2,699							1										1
Total	3	8	15	32	41	36	20	9	9	2	5	1	1			1	183

$r = +0.1887 \pm 0.0481$.

TABLE 18.—*Correlation between viscosity of one concentration of flour and water and loaf volume of hard red spring wheat flours, crop of 1923*

Loaf volume in cubic centimeters	Number of samples according to viscosity in degrees MacMichael											
	125–149	150–174	175–199	200–224	225–249	250–274	275–299	300–324	325–349	350–374	375–399	Total
1,800 to 1,899		1		1		1						3
1,900 to 1,999			2	3	2			1				8
2,000 to 2,099	2			6	1	2	2					13
2,100 to 2,199		2	2	9	12	6	7	8	1			47
2,200 to 2,299			2	3	7	5	4	3				24
2,300 to 2,399			2	3		5	2	3		1	1	17
2,400 to 2,499					1	7			1			9
2,500 to 2,599				1		1	1	1				4
2,600 to 2,699						1		1				2
2,700 to 2,799							1					1
Total	2	3	8	26	23	28	17	17	2	1	1	128
$r = +0.3260 \pm 0.0533$.												

It will be seen from an examination of Table 18 that there was a significant relationship existing between the one concentration or absolute viscosity of the flour-and-water suspension of the hard red spring wheat flour under the same conditions of hydrogen-ion concentration and the baking strength as interpreted by loaf volume. The coefficient in this series of samples was $+0.3260 \pm 0.0533$.

Although the coefficient obtained with the hard red winter wheat samples was not large, namely, $+0.1887 \pm 0.0481$, it is likewise significant, inasmuch as it is almost four times the probable error.

When differences in the texture of the various loaves of bread were taken into consideration these coefficients changed somewhat. The samples of winter wheat flour gave a coefficient of $+0.1643 \pm 0.0485$, as compared with $+0.1887 \pm 0.0481$, whereas for samples of spring wheat flour the coefficient changed from $+0.3260 \pm 0.0533$ to $+0.3384 \pm 0.0528$.

These coefficients are larger than those obtained when texture of kernel was taken as an index of gluten quality, and they are decidedly larger than the coefficients obtained when the water-absorbing power of the flours was studied as an index of gluten quality. They are, however, much lower in value than the coefficients obtained from a study of either the protein content of the wheat flour as indices of gluten quality.

Because of the encouraging results obtained with the one concentration of flour and water on the samples of the 1923 crop, the viscometric study was enlarged when applied to the 1924 crop. On the samples for that crop, in addition to the one-concentration test the quality angle *b* of Gortner and Sharp (*17*), the viscosity of 2 gm. of protein, and the viscosity of 1 per cent of protein were compared with the associated loaf volumes. The only change in the technique as described above was that the flour-and-water suspension was acidified by means of one-half c. c. of 20 per cent lactic acid, according to the recommendations of Gortner (*9*). Tests were made by the four methods mentioned above on 116 samples of hard red spring wheat and 76 samples of hard red winter wheat of the crop of 1924.

As before, when the one-concentration method was used with the hard red spring flour a significant correlation of $+0.3310 \pm 0.0558$

was obtained, this coefficient changing to $+0.2112 \pm 0.0598$ when the texture of the crumb was taken into account. Again, as with the 1923 crop, only a slight relationship existed between the one-concentration viscosity values of the 76 samples of hard red winter wheat and the volume of the baked loaf, the coefficient of correlation being $+0.0745 \pm 0.0774$. Neither of the above coefficients was improved upon by being corrected for the texture of the crumb.

ANGLE *b* AS AN INDEX OF BAKING STRENGTH

A comparison of angle *b* with loaf volume as determined on 114 samples of the 1924 hard red spring wheat crop and on 76 samples of the 1924 hard red winter wheat crop are given in Tables 19 and 20.

From the 114 samples of hard red spring wheat flour, little relationship was apparent when angle *b* of Gortner was determined and the values correlated with the associated loaf volume, the coefficient of correlation being -0.0320 ± 0.0631. For the 76 samples of hard red winter wheat flour a fairly high coefficient was found when angle *b* was compared with the associated loaf volume, namely, $+0.2214 \pm 0.0736$.

TABLE 19.—*Correlation between quality angle b and loaf volume of hard red spring wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to range in tangent of Gortner angle b											
	1.30–1.44	1.45–1.59	1.60–1.74	1.75–1.89	1.90–2.04	2.05–2.19	2.20–2.34	2.35–2.49	2.50–2.64	2.65–2.79	2.80–2.94	Total
1,900 to 1,999			2	5		1	1					9
2,000 to 2,099		1	3	2	3	1	1	2		1		14
2,100 to 2,199	1	1	4	4	7	7	3	1	1	1		30
2,200 to 2,299	1		1	1	3	6	3	1	2			18
2,300 to 2,399		2	1	4	2	1	5	1	1		1	18
2,400 to 2,499	1			2	1		1			1		6
2,500 to 2,599			2		1	1	1	1				6
2,600 to 2,699	1	1		1	1		1					5
2,700 to 2,799	1			1	1			1				4
2,800 to 2,899	1				1							2
2,900 to 2,999									1			1
3,000 to 3,099				1								1
Total	6	5	13	21	20	17	16	7	5	3	1	114

$r = -0.0320 \pm 0.0631$.

TABLE 20.—*Correlation between quality angle b and loaf volume of hard red winter wheat flours, crop of 1924*

Loaf volume in cubic centimeters	Number of samples according to range in tangent of Gortner angle b											
	1.60–1.74	1.75–1.89	1.90–2.04	2.05–2.19	2.20–2.34	2.35–2.49	2.50–2.64	2.65–2.79	2.80–2.94	2.95–3.09	3.10–3.24	3.25 and more	Total
1,600 to 1,699				1							1		2
1,700 to 1,799		1	1										2
1,800 to 1,899	5	2	3	1	2								13
1,900 to 1,999		4	6	1	2	1	1			1		1	17
2,000 to 2,099	2	2	2	1	3		1				2		13
2,100 to 2,199		3	1	5	2	1	2	1					15
2,200 to 2,299				2	2	2			1	1			8
2,300 to 2,399						1				1			2
2,400 to 2,499			1										1
2,500 to 2,599				2		1							3
Total	7	12	14	13	11	6	4	1	1	3	3	1	76

$r = +0.2214 \pm 0.0736$.

VISCOSITY OF 2 GRAMS OF PROTEIN AND LOAF VOLUME

Since it has been shown that flour starch under the conditions of the Gortner and Sharp test (*17*) has but little influence upon the viscosity, it has been suggested that when the total protein content of the flour sample is known, single concentrations of flour could be prepared in such a way as to make possible viscometric measurements on the same amount of protein. This was done with 113 samples of hard red spring wheat flour and with 73 samples of hard red winter wheat flour of the 1924 crop. No distribution tables are given. Suffice it to say that the coefficient of correlation obtained by comparing the 73 samples of hard red winter wheat flour with the associated loaf volume was $+0.0409 \pm 0.0788$, whereas from the 113 samples of hard red spring wheat flour a coefficient of -0.0251 ± 0.0634 was obtained.

VISCOSITY OF 1 PER CENT OF PROTEIN AND LOAF VOLUME

It has likewise been suggested that viscosity readings may be reduced to a common protein basis by dividing the viscosity readings by the protein content of the flour. Coefficients of correlation resulting from a comparison of such values with the associated baking tests were of no practical significance.

OTHER CORRELATION STUDIES

In addition to the correlations measuring gluten quality, the relationships existing between the physical and chemical factors were compared. Texture of wheat, protein content of wheat, water-absorbing power of flour, and viscosity determinations in their various forms were compared with each other for each class of wheat for the two crop years. These data are given in Tables 21 and 22.

The relationship existing between texture of the kernel; that is, the percentage of dark, hard, and vitreous kernels in the wheat, and other tests was first considered.

The degree of relationship found between the protein content of the wheat and kernel texture was high. In each crop year and in both classes of wheat there were also fairly significant relationships existing between the texture of kernel and the water-absorbing power of the flour and between the texture of the kernel and the viscosity of one concentration of flour and water. The coefficient from the latter determination was just as high in most instances as was the coefficient of correlation between the kernel texture and the protein content of the wheat. When the Gortner angle *b* (*9*) was considered, the relationship between it and the texture of the kernel was small.

The protein content of the flour was next compared with the water-absorbing power of the flour, with the viscosity of one concentration of flour and water, with the Gortner angle *b*, and with the percentage of wet and dry gluten in the flour.

TABLE 21.—*Correlations between the kernel texture of wheat and other tests used to express gluten quality*

Tests	Number of samples	Percentage of dark, hard, and vitreous kernels			Comparative test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
1923:									
Hard red winter wheat—									
Texture of wheat and protein content	183	67.5	99.9	6.0	[a] 12.0	[a] 15.90	[a] 8.20	+0.6004	±0.0319
Texture of wheat and water-absorbing power	183	67.5	99.9	6.0	[a] 72.9	[a] 81.70	[a] 66.90	+.4190	±.0411
Texture of wheat and viscosity of one concentration of flour and water	183	67.5	99.9	6.0	[b] 226	[b] 476	[b] 108	+.6776	±.0270
Hard red spring wheat—									
Texture of wheat and protein content	128	81.0	100.0	25.2	[a] 12.65	[a] 15.20	[a] 9.20	+.6414	±.0351
Texture of wheat and water-absorbing power	128	81.0	100.0	25.2	[a] 71.70	[a] 84.00	[a] 64.80	+.3928	±.0504
Texture of wheat and viscosity of one concentration of flour and water	128	81.0	100.0	25.2	[b] 252	[b] 388	[b] 128	+.5597	±.0409
1924:									
Hard red winter wheat—									
Texture of wheat and protein content	76	78.4	100.0	18.2	[a] 12.90	[a] 18.00	[a] 8.40	+.5593	±.0532
Texture of wheat and water-absorbing power	76	78.4	100.0	18.2	[a] 73.60	[a] 80.80	[a] 66.60	+.4605	±.0610
Texture of wheat and viscosity of one concentration of flour and water	75	78.1	100.0	18.2	[b] 208	[b] 417	[b] 72	+.3166	±.0701
Texture of wheat and Gortner angle *b*	76	78.4	100.0	18.2	[c] 2.18	[c] 3.69	[c] 1.64	−.2065	±.0741
Hard red spring wheat—									
Texture of wheat and protein content	113	89.4	99.9	18.0	[a] 13.70	[a] 18.80	[a] 9.50	+.3981	±.0536
Texture of wheat and water-absorbing power	116	89.8	99.9	18.0	[a] 73.80	[a] 81.00	[a] 66.80	+.3824	±.0535
Texture of wheat and viscosity of one concentration of flour and water	113	89.5	99.9	18.0	[b] 238	[b] 423	[b] 98	+.3537	±.0555
Texture of wheat and Gortner angle *b*	116	89.8	99.9	18.0	[c] 2.00	[c] 2.81	[c] 1.27	+.0102	±.0626

[a] Per cent. [b] Degrees MacMichael. [c] Tangent of angle *b*.

TABLE 22.—*Correlations between various tests used to express gluten quality*

Tests	Crop year	Class of wheat	Number of samples	Percentage of independent factor			Dependent-factor determinations			Coefficient of correlation	Probable error
				Average	Maximum	Minimum	Average	Maximum	Minimum		
Protein content of flour and viscosity of one concentration of flour and water	1923	Hard red winter	183	16.89	15.68	7.30	[a]226	[a]476	[a]108	+0.7449	±0.0222
Do	1923	Hard red spring	128	11.71	15.10	8.32	[a]252	[a]388	[a]128	+.7344	±.0275
Do	1924	Hard red winter	116	12.99	17.29	8.47	[a]238	[a]423	[a]98	+.5307	±.0450
Do	1924	Hard red spring	75	11.80	17.10	6.78	[a]208	[a]417	[a]72	+.6297	±.0470
Protein content of flour and water-holding capacity of flour	1923	Hard red winter	183	10.89	15.68	7.30	[b]72.9	[b]81.7	[b]66.9	+.4327	±.0450
Do	1923	Hard red spring	128	11.71	15.10	8.32	[b]71.7	[b]84.0	[b]65.6	+.1381	±.0585
Do	1924	do	117	12.99	17.29	8.47	[b]73.8	[b]81.0	[b]66.8	+.4293	±.0509
Do	1924	Hard red winter	76	11.80	17.10	6.78	[b]73.6	[b]80.8	[b]66.6	+.5813	±.0512
Protein content of wheat and Gortner angle *b*	1924	do	76	12.99	18.00	8.40	[c]2.18	[c]3.69	[c]1.64	−.3764	±.0664
Do	1924	Hard red spring	116	13.70	18.80	9.50	[c]2.00	[c]2.81	[c]1.27	+.0512	±.0625
Protein content of wheat flour and Gortner angle *b*	1924	Hard red winter	71	11.49	16.70	7.52	[c]2.21	[c]3.69	[c]1.64	+.1584	±.0780
Do	1924	Hard red spring	121	12.88	17.29	8.47	[c]2.00	[c]2.80	[c]1.27	−.0150	±.0613
Water-holding capacity and Gortner angle *b*	1924	Hard red winter	76	73.60	80.80	66.60	[c]2.18	[c]3.69	[c]1.64	−.3863	±.0658
Do	1924	Hard red spring	117	73.80	81.00	66.80	[c]2.00	[c]2.81	[c]1.27	+.1020	±.0617
Water-holding capacity and viscosity of one concentration of flour and water	1923	Hard red winter	183	72.90	81.70	66.90	[a]226	[a]476	[a]108	+.5620	±.0341
Do	1923	Hard red spring	128	64.80	8.40	71.10	[a]252	[a]388	[a]128	+.1739	±.0578
Do	1924	Hard red winter	75	73.60	80.80	66.60	[a]208	[a]417	[a]72	+.5590	±.0535
Do	1924	Hard red spring	116	73.80	81.00	66.80	[a]238	[a]423	[a]98	+.3267	±.0559
Percentage of wet gluten and protein content of flour	1923	do	106	12.24	15.50	9.80	[b]35.71	[b]49.36	[b]25.10	+.8643	±.0166
Do	1923	Hard red winter	119	11.04	15.68	8.61	[b]33.78	[b]52.12	[b]22.18	+.8988	±.0119
Protein content of wheat and percentage of dry gluten	1923	do	118	12.37	16.79	9.82	[b]11.87	[b]17.29	[b]8.52	+.8920	±.0127
Do	1923	Hard red spring	82	12.79	14.95	10.84	[b]13.00	[b]15.76	[b]10.27	+.7551	±.0320
Protein content of flour and percentage of wet gluten	1923	Hard red winter	119	11.04	15.68	8.61	[b]33.78	[b]52.12	[b]22.18	+.8988	±.0119
Do	1923	Hard red spring	106	12.24	15.50	9.80	[b]35.71	[b]49.36	[b]25.10	+.8643	±.0166
Protein content of flour and percentage of dry gluten	1923	Hard red winter	118	11.24	14.65	8.64	[b]11.89	[b]17.29	[b]8.58	+.9166	±.0099
Do	1923	Hard red spring	82	12.13	14.25	9.80	[b]13.00	[b]15.76	[b]10.27	+.8473	±.0210
Percentage of wet gluten and viscosity of one concentration of flour and water	1923	do	106	35.71	49.36	25.10	[a]245	[a]346	[a]151	+.1860	±.0633
Do	1923	Hard red winter	119	33.78	52.12	22.18	[a]222	[a]373	[a]108	+.7451	±.0275
Protein content of wheat and protein content of flour	1923	Hard red spring	128	12.65	15.20	9.20	11.71	15.10	8.32	+.9388	±.0071

[a] Degrees MacMichael.

[b] Per cent.

[c] Tangent of angle *b*.

A high and positive correlation was found between the protein content of the flour and the water absorption in every case with the exception of spring wheat flours of the 1923 crop. There was also a high and positive correlation between the protein content of the flour and the absolute or one concentration viscosity test for both classes of wheat in each crop year. When flour protein was compared with angle *b* the relationship was low with the hard red winter wheat crop of 1924 and of no significance with the samples of flour from the 1924 hard red spring-wheat crop.

The correlation between the protein of flour and the percentage of dry gluten was almost perfect. A coefficient of $+0.9166 \pm 0.0099$ was obtained from the 1923 hard red winter wheat flour and one of $+0.8473 \pm 0.0210$ for 82 samples of flour of the 1923 hard red spring wheat crop. High coefficients were also obtained when the protein of the flour was compared with the percentage of wet gluten in the same samples tested for dry gluten content, $+0.8988 \pm 0.0119$ being the coefficient obtained from a study of the 119 samples of hard red winter flour and $+0.8643 \pm 0.0166$, that for the hard red spring wheat flour.

The relation existing between gluten quality angle *b* and the protein content of the wheat is of no significance, nor is the relationship found between the water-absorbing power of the flour and quality angle *b*.

In three out of four instances a high and significant relation was found between the absolute one-concentration test and the water-absorbing power of the flour.

High correlation coefficients were likewise found between the protein content of the wheat and the texture of the kernel, between the protein content of the flour and the water-absorbing power of the flour, between the viscosity of a single concentration of the flour and water and the protein of the flour, and between the percentage of wet and dry gluten and the protein content of the flour.

The substitution of one-concentration viscosity values for the protein in the flour resulted in a coefficient somewhat lower in most instances than that found to exist between the protein content of flour and the associated loaf volume. Thus it becomes apparent from a study of the distribution tables that the one-concentration viscosity test is a measure of the quantity of protein in wheat flour rather than an index of the gluten quality, if the baking test is used as the measure of gluten quality.

Why higher correlation values were not forthcoming, especially from the use of some of the well-known tests, such as the water-absorbing power of the flour or kernel texture of the wheat, it is difficult to say. Gortner (*9*) criticizes baking technique in general because the majority of baking experiments are not so conducted as to insure the presence of an adequate amount of diastase in all instances. Alsberg (*1*) objects to the use of only one baking method for studying the gluten quality of wheat flour, arguing that several trials should be made with the same flour under different baking conditions before final judgment is passed on the baking quality of the flour. Possibly if an adequate supply of diastase was at hand or if several bakings were made, using different formulas, a different set of coefficients would be obtained. Blish (*4*), however, baked

flours with adequate diastatic qualities and found no answer to the gluten-quality problem even when this factor was considered.

On the other hand, tests made in connection with other investigations show that a change in baking procedure will affect the correlations. Eighty-four samples of the 1923 hard red winter wheat flour and 39 samples of the 1923 hard red spring wheat flour were baked in another laboratory and by another baker. The results obtained by this outside laboratory were compared with the bakings made of the identical flours in the milling and baking laboratory of the Bureau of Agricultural Economics. The baking data from each laboratory were compared with the protein content of the flour, and the following correlation coefficients were obtained. With the 39 samples of hard red spring wheat the coefficient of correlation obtained from the data submitted by the outside laboratory was $+0.2757 \pm 0.0998$, whereas from the data submitted by the milling and baking laboratory the coefficient was $+0.4405 \pm 0.0870$. A comparison of the baking data of the 84 samples of hard red winter wheat flour with their crude-protein contents showed a coefficient of $+0.3357 \pm 0.0653$ in the work of the outside laboratory and one of $+0.3974 \pm 0.0620$ in the milling and baking laboratory.

It is apparent, then, that before a set of results can be obtained that will be standard for all laboratories, the recommendation of Fitz (7) relative to a standard baking test will have to be seriously considered.

In Tables 23, 24, 25, and 26 is given a summary of the coefficients obtained from the various tests for gluten quality.

TABLE 23.—*Correlations between loaf volume and physical and chemical tests of gluten quality of hard red winter wheat flours, crop of 1923*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	183	2,129	2,695	1,770	[a] 67.5	[a] 99.9	[a] 6.00	+0.0990	±0.0494
Protein content of wheat	183	2,129	2,695	1,770	[a] 12.00	[a] 15.90	[a] 8.20	+.4281	±.0407
Protein content of flour	183	2,129	2,695	1,770	[a] 10.89	[a] 15.68	[a] 7.30	+.4249	±.0409
Percentage of dry gluten in flour	118	2,169	2,695	1,885	[a] 11.89	[a] 17.29	[a] 8.53	+.4151	±.0514
Percentage of wet gluten in flour	119	2,169	2,695	1,885	[a] 33.78	[a] 52.12	[a] 22.18	+.4806	±.0475
Water-absorbing power of flour	183	2,129	2,695	1,770	[b] 60.1	[b] 74.7	[b] 53.5	−.0358	±.0498
Water-holding capacity of flour	183	2,129	2,695	1,770	[b] 72.9	[b] 81.7	[b] 66.9	−.0289	±.0498
Viscosity of 1 concentration of flour and water	183	2,129	2,695	1,770	[c] 226	[c] 476	[c] 108	+.1887	±.0481

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Kernel texture of wheat	183	1,913	2,533	1,561	[a] 67.5	[a] 99.9	[a] 6.00	+0.1027	±0.0493
Protein content of wheat	183	1,913	2,533	1,561	[a] 12.00	[a] 15.90	[a] 8.20	+.3932	±.0422
Portein content of flour	183	1,913	2,533	1,561	[a] 10.89	[a] 15.68	[a] 7.30	+.3816	±.0426
Percentage of dry gluten in flour	118	1,953	2,533	1,649	[a] 11.89	[a] 17.29	[a] 8.58	+.4181	±.0512
Percentage of wet gluten in flour	119	1,953	2,533	1,649	[a] 33.78	[a] 52.12	[a] 22.18	+.4792	±.0476
Water-holding capacity of flour	183	1,913	2,533	1,561	[b] 72.9	[b] 81.7	[b] 66.9	−.0711	±.0496
Viscosity of 1 concentration of flour and water	183	1,913	2,533	1,561	[c] 226	[c] 476	[c] 108	+.1643	±.0485

[a] Per cent. [b] Cubic centimeters. [c] Degrees MacMichael.

TABLE 24.—*Correlations between loaf volume and physical and chemical tests of gluten quality of hard red spring wheat flours, crop of 1923*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	128	2,268	2,725	1,830	[a] 81.00	[a] 100.00	[a] 25.20	+0.2612	±0.0556
Protein content of wheat	128	2,268	2,725	1,830	[a] 12.65	[a] 15.20	[a] 9.20	+.4610	±.0470
Protein content of flour	128	2,268	2,725	1,830	[a] 11.71	[a] 15.10	[a] 8.32	+.4641	±.0468
Percentage of dry gluten in flour	82	2,212	2,500	1,870	[a] 13.00	[a] 15.76	[a] 10.27	+.2271	±.0796
Percentage of wet gluten in flour	106	2,183	2,525	1,840	[a] 35.71	[a] 49.36	[a] 25.10	+.1355	±.0643
Water-absorbing power of flour	128	2,268	2,725	1,830	[b] 58.60	[b] 71.50	[b] 52.90	+.0187	±.0896
Water-holding capacity of flour	128	2,268	2,725	1,830	[b] 71.10	[b] 84.00	[b] 64.80	−.0164	±.0596
Viscosity of 1 concentration of flour and water	128	2,268	2,725	1,830	[c] 252	[c] 388	[c] 128	+.3260	±.0533

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Kernel texture of wheat	128	1,957	2,540	1,624	[a] 81.00	[a] 100.00	[a] 25.20	+0.2937	±0.0545
Protein content of wheat	128	1,957	2,540	1,624	[a] 12.65	[a] 15.20	[a] 9.20	+.4068	±.0498
Protein content of flour	128	1,957	2,540	1,624	[a] 11.71	[a] 15.10	[a] 8.32	+.3856	±.0508
Percentage of dry gluten in flour	82	1,962	2,250	1,710	[a] 13.00	[a] 15.76	[a] 10.27	+.0822	±.0740
Percentage of wet gluten in flour	106	1,947	2,280	1,656	[a] 35.71	[a] 49.36	[a] 25.10	+.1577	±.0639
Water-holding capacity of flour	128	1,957	2,540	1,624	[b] 71.10	[b] 84.00	[b] 64.80	+.0965	±.0591
Viscosity of 1 concentration of flour and water	128	1,957	2,540	1,624	[c] 252	[c] 388	[c] 128	+.3384	+.0528

[a] Per cent. [b] Cubic centimeters. [c] Degrees MacMichael.

TABLE 25.—*Correlations between loaf volume and physical and chemical tests for gluten quality of hard red spring wheat flours, crop of 1924*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	113	2,276	2,970	1,920	[a] 89.50	[a] 99.90	[a] 18.00	+0.1517	±0.0620
Protein content of wheat	116	2,286	2,970	1,920	[a] 13.70	[a] 18.80	[a] 9.50	+.4184	±.0547
Protein content of flour	116	2,286	2,970	1,920	[a] 12.99	[a] 17.29	[a] 8.47	+.4005	±.0526
Water-absorbing power of flour	117	2,286	2,970	1,920	[b] 60.20	[b] 67.40	[b] 52.90	+.0841	±.0823
Water-holding capacity of flour	117	2,286	2,970	1,920	[b] 73.80	[b] 81.00	[b] 66.80	+.0556	±.0822
Viscosity of 1 concentration of flour and water	116	2,286	2,970	1,920	[c] 238	[c] 423	[c] 98	+.3310	±.0558
Viscosity of 2 grams of protein	113	2,042	2,580	1,640	[c] 146	[c] 277	[c] 52	−.0070	±.0635
Viscosity, Gortner angle [b]	114	2,270	2,970	1,920	[d] 2.00	[d] 2.81	[d] 1.27	−.0320	±.0631

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Kernel texture of wheat	113	2,044	2,909	1,416	[a] 89.50	[a] 99.90	[a] 18.00	+0.1249	±0.0625
Protein content of wheat	116	2,039	2,909	1,418	[a] 13.70	[a] 18.80	[a] 9.50	+.3694	±.0546
Protein content of flour	116	2,039	2,909	1,418	[a] 12.99	[a] 17.29	[a] 8.47	+.3278	±.0559
Water-holding capacity of flour	117	2,039	2,909	1,418	[b] 73.80	[b] 81.00	[b] 66.80	+.1076	±.0816
Viscosity of 1 concentration of flour and water	116	2,039	2,909	1,418	[c] 238	[c] 423	[c] 98	+.2112	±.0598
Viscosity of 2 grams of protein	113	1,806	2,394	920	[c] 146	[c] 277	[c] 52	−.0251	±.0634
Viscosity, Gortner angle *b*	114	2,044	2,909	1,418	[d] 2.00	[d] 2.81	[d] 1.27	−.0277	±.0631

[a] Per cent. [b] Cubic centimeters. [c] Degrees MacMichael. [d] Tangent of the angle *b*.

TABLE 26.—*Correlations between loaf volume and physical and chemical tests of gluten quality of hard red winter wheat flours, crop of 1924*

TESTS IN WHICH DETERMINATIONS WERE NOT CORRECTED FOR CRUMB TEXTURE

Test	Number of samples	Loaf volume, c. c.			Quality test determinations			Coefficient of correlation	Probable error
		Average	Maximum	Minimum	Average	Maximum	Minimum		
Kernel texture of wheat	76	2,041	2,580	1,640	[a] 78.4	[a] 100.00	[a] 18.20	+0.0287	±0.0773
Protein content of wheat	76	2,041	2,580	1,640	[a] 12.90	[a] 18.00	[a] 8.40	−.0370	±.0773
Protein content of flour	76	2,041	2,580	1,640	[a] 11.80	[a] 17.10	[a] 6.78	+.1153	±.0763
Water-absorbing power of flour	76	2,041	2,580	1,640	[b] 60.20	67.70	[b] 52.90	−.3045	±.0702
Water-holding capacity of flour	76	2,041	2,580	1,640	[b] 73.60	[b] 80.80	[b] 66.60	−.3134	±.0698
Viscosity of 1 concentration	76	2,041	2,580	1,640	[c] 208	[c] 417	[c] 72	+.0745	±.0774
Viscosity of 2-gram sample	73	2,260	3,030	1,920	[c] 135	[c] 262	[c] 58	+.0409	±.0788
Viscosity, Gortner angle *b*	76	2,041	2,580	1,640	[d] 2.18	[d] 3.69	[d] 1.64	+.2214	±.0736

TESTS IN WHICH DETERMINATIONS WERE CORRECTED FOR CRUMB TEXTURE

Kernel texture of wheat	76	1,802	2,394	927	[a] 78.40	[a] 100.00	[a] 18.20	+0.0328	±0.0773
Protein content of wheat	76	1,802	2,394	927	[a] 12.90	[a] 18.00	[a] 8.40	−.0353	±.0773
Protein content of flour	76	1,802	2,394	927	[a] 11.80	[a] 17.10	[a] 6.78	+.0464	±.0772
Water-holding capacity of flour	76	1,802	2,394	927	[b] 73.60	[b] 80.60	[b] 66.60	−.2613	±.0721
Viscosity of 1 concentration	76	1,802	2,394	927	[c] 208	[c] 417	[c] 72	+.0550	±.0777
Viscosity of 2-gram sample	73	2,029	2,909	1,418	[c] 135	[c] 262	[c] 58	−.0377	±.0778
Viscosity, Gortner angle *b*	76	1,802	2,394	927	[d] 2.18	[d] 3.69	[d] 1.64	+.1246	±.0736

[a] Per cent. [b] Cubic centimeters. [c] Degrees MacMichael. [d] Tangent of angle *b*.

CONCLUSIONS

Although there were slight differences in the order of merit, dependent on the class of wheat and the crop year, it appears from the data submitted that a crude-protein determination, either on the wheat or on the flour milled from this wheat, is the best single-factor test of gluten quality.

Next in order of merit comes the washed-gluten test. This test gives remarkably good results in the hands of a single operator, but the results obtained are not easily reproducible. This is not true of the crude-protein test.

Among the newer viscosity tests for determining baking strength, the single-concentration test is the only one that has given any satisfaction. Compared with the other tests reported in this investigation, this type of viscosity test is nearly as good as the dry-gluten or wet-gluten test and decidedly better than a determination of kernel texture or a determination of the water-absorbing power of the flour.

Tests of the kernel texture and the water-absorbing power of flour did not give very high coefficients of correlation. These tests rank as about equal in choice as tests for gluten quality.

As a measure of baking strength, the four-point system of Gortner and Sharp (*17*), from which the angle *b* (*9*) is calculated, has proved of negative value as a means of predicting baking strength. The same is true of tests of the viscosity of an even amount of protein or the viscosity of an even percentage of protein as a measure of gluten quality.

The lack of closer agreement between the results of the newer gluten-quality tests and the volume of the baked loaf is, no doubt, partly due to variations in baking procedure.

LITERATURE CITED

(1) ALSBERG, C. L.
1924. THE COMING WHEAT DEFICIENCY. Baking Technol. 3: 54–58.

(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1920. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. REVISED TO NOV. 1, 1919. 417 p., illus. Washington, D. C.

(3) BAILEY, C. H.
1924. REPORT OF THE EXPERIMENTAL WORK, STATE TESTING MILL CROP SEASON OF 1922. Minn. Dept. Agr. Bul. 34, 29 p., illus.

(4) BLISH, M. J., and SANDSTEDT, R. M.
1925. VISCOSITY STUDIES WITH NEBRASKA WHEAT FLOURS. Cereal Chem. 2: 191–201.

(5) COLEMAN, D. A., FELLOWS, H. C., and DIXON, H. B.
1925. A STUDY OF METHODS FOR MAKING PROTEIN TESTS ON WHEAT. Cereal Chem. 2: 132–164.

(6) DURHAM, R. K.
1925. EFFECT OF HYDROGEN PEROXIDE ON RELATIVE VISCOSITY MEASUREMENTS OF WHEAT AND FLOUR SUSPENSIONS. Cereal Chem. 2: 297–305.

(7) FITZ, L. A.
1925. REPORT OF COMMITTEE ON STANDARD FORMULA AND METHOD OF PROCEDURE FOR EXPERIMENTAL BAKING TESTS. Cereal Chem. 2: 232–235.

(8) FRANK, W. L.
1923. PROTEIN CONTENT VS. TEXTURE VS. KERNEL WEIGHT IN SOUTHWESTERN MARKET-RUN HARD WINTER WHEAT. Mod. Miller 50 (28): 23, 34.

(9) GORTNER, R. A.
1924. VISCOSITY AS A MEASURE OF GLUTEN QUALITY. Cereal Chem. 1: 75–81, illus.

(10) HALL, F. P.
1923. METHODS OF MEASURING THE PLASTICITY OF CLAYS. U. S. Dept. Com., Bur. Standards Technol. Papers 234, p. 345–366, illus.

(11) HERSCHEL, W. H.
1920. THE MACMICHAEL TORSIONAL VISCOSIMETER. Jour. Indus. and Engin. Chem. 12: 282–286, illus.

(12) MANGELS, C. E.
1925. PROTEIN CONTENT OF NORTH DAKOTA WHEAT. N. Dak. Agr. Expt. Sta. Bul. 191, 41 p., illus.

(13) ——— and SANDERSON, T.
1925. THE CORRELATION OF THE PROTEIN CONTENT OF HARD RED SPRING WHEAT WITH PHYSICAL CHARACTERISTICS AND BAKING QUALITY. Cereal Chem. 2: 107–112.

(14) MELLOR, J. W.
1913. HIGHER MATHEMATICS FOR STUDENTS OF CHEMISTRY AND PHYSICS. Ed. 4, enl., 641 p., illus. London, New York [etc.]

(15) MILLS, F. C.
[1925]. STATISTICAL METHODS APPLIED TO ECONOMICS AND BUSINESS. 604 p., illus. New York.

(16) MORGAN, R. W.
1924. A PRACTICAL APPLICATION OF THE VISCOSIMETER TO THE MILL. Cereal Chem. 1: 288–292.

(17) SHARP, P. F., and GORTNER, R. A.
1923. VISCOSITY AS A MEASURE OF HYDRATION CAPACITY OF WHEAT FLOUR AND ITS RELATION TO BAKING STRENGTH. Minn. Agr. Expt. Sta. Tech. Bul. 19, 119 p., illus.

(18) SHERWOOD, R. C.
1924. REPORT OF SURVEY OF 1924 MINNESOTA WHEAT CROP. Minn. Dept. Agr. Bul. 36, 11 p., illus.

(19) ———
1925. PROTEIN SURVEY OF 1925 MINNESOTA WHEAT CROP. Minn. Dept. Agr. Bul. 45, 15 p., illus.

(20) SHOLLENBERGER, J. H., and COLEMAN, D. A.
1926. RELATION OF KERNEL TEXTURE TO THE PHYSICAL CHARACTERISTICS, MILLING AND BAKING QUALITIES, AND CHEMICAL COMPOSITION OF WHEAT. U. S. Dept. Agr. Bul. 1420, 16 p., illus.

(21) Shollenberger, Marshall, W. K., and Coleman, D. A.
1924. EXPERIMENTAL MILLING AND BAKING, INCLUDING CHEMICAL DETERMINATIONS. U. S. Dept. Agr. Bul. 1187, 53 p., illus.
(22) Smith, E. E.
1925. VISCOSITY AND BAKING QUALITY. Cereal Chem. 2: 177–190, illus.
(23) Stockham, W. L.
1920. SOME FACTORS RELATED TO THE QUALITY OF WHEAT AND STRENGTH OF FLOUR. N. Dak. Agr. Expt. Sta. Bul. 139, 69 p., illus.
(24) Thomas, L. M.
1917. A COMPARISON OF SEVERAL CLASSES OF AMERICAM WHEATS AND A CONSIDERATION OF SOME FACTORS INFLUENCING QUALITY. U. S. Dept. Agr. Bul. 557, 28 p., illus.
(25) Weaver, H. A., and Goldtrap, W. A.
1922. FLOUR STRENGTH. Jour. Amer. Assoc. Cereal Chem. 7: 115–123.
(26) Zinn, J.
1923. CORRELATIONS BETWEEN VARIOUS CHARACTERS OF WHEAT AND FLOUR AS DETERMINED FROM PUBLISHED DATA FROM CHEMICAL, MILLING, AND BAKING TESTS OF A NUMBER OF AMERICAN WHEATS. Jour. Agr. Research 23: 529–548.

THE LIFE HISTORY, HABITS, AND ECONOMIC IMPORTANCE OF SOME MONONCHS[1]

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INTRODUCTION

The relationships existing, under actual field conditions, between the predacious nemas of the genus Mononchus and the economically important plant-parasitic nemas is a subject upon which published information is exceedingly meager. There is likewise but little information concerning the factors which may influence these relationships. In view of these facts, the studies here described were begun for the purpose of learning, if possible, what mononchs were present in the soils of Utah and Idaho fields infested with the sugar-beet nema, *Heterodera schachtii* Schmidt, and what the possibilities were of their being, or becoming, of economic importance in the control of this highly destructive pest.

At the same time it was imperative that data be collected on the factors which might influence the mononchs in their habits and thus directly or indirectly have a bearing on the number in the soil. The factors immediately suggested were: Temperature, moisture, food supply, and diseases or enemies. Doubtless these are the four most important factors in nemic life, any one of which may vitally influence the activities and number of mononchs.

METHODS

Mononch colonies were located by making about 200 examinations of soil from fields infested with *Heterodera schachtii*. These fields were distributed over practically all the sugar-beet growing sections of Utah and southern Idaho. The soils examined varied from the heavy clay loams of the lower-valley fields to the light sandy soils of the upper-valley levels. From these examinations it was possible to obtain fairly accurate data on the species of mononchs present and the numbers in which they occurred in the various fields.

The soil samples were examined in the laboratory, using the methods devised by Cobb.[3] It was found that the mononch population of the fields varied greatly. In some of the heavy clay loam soils no mononchs were found, while in the lighter sandy loams as many as four species were present. The populations of the various species ranged from only a few specimens to nearly 300,000,000 per acre.

In 1923 two fields were selected which apparently offered the most favorable prospects because of the number of mononchs present and the degree of *Heterodera schachtii* infestation. These two

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[2] The writer is indebted to L. A. Giddings, Charles Price, and S. E. Aldous for assistance in this work.
[3] COBB, N. A. ESTIMATING THE NEMA POPULATION OF SOIL, WITH SPECIAL REFERENCE TO THE SUGAR-BEET AND ROOT-GALL NEMAS. U. S. Dept. Agr., Bur. Plant Indus., Off. Agr. Tech. Circ. 1, 48 p., illus. 1918.

fields contained colonies of all the species of mononchs found in the cultivated areas of Utah, making it possible to obtain a maximum amount of data with a minimum of labor.

To determine if there was any increase in the mononch population correlated with the numbers of *Heterodera schachtii* present, arrangements were made with the owners of these fields to plant the latter with sugar beets every year. This arrangement insured a large population of *H. schachtii*, upon which the mononchs could feed if they were inclined to do so.

DESCRIPTION OF FIELDS SELECTED

Field No. 1—Marion Bullen Farm, Lewiston, Utah

Sugar beets had been grown in the field on the Bullen farm almost continuously for about 20 years, and it had become so severely infested with *Heterodera schachtii* that the crop was practically a failure. The soil is a light sandy loam of loose texture, typical of the Lewiston section. In general, the soil is run down because of overcropping with beets and lack of manure. It is irrigated by the "subbing" method; that is, by making large ditches 50 to 100 feet apart, which are kept filled with water until the intervening spaces are completely saturated. However, this field lies alongside a large canal, and it was not necessary to apply any water in addition to that which leached into it from the canal. This condition, it might be expected, would give a more uniform distribution of moisture from month to month than would be found in a field irrigated by the furrow method, where the sudden application of water would quickly saturate the upper soil but would be followed by almost immediate drying out.

Two mononchs were found inhabiting this field: *Mononchus macrostoma* Bastian, 1866, and *M. parabrachyurus* Thorne, 1924.

Field No. 2—A. W. Davis Farm, Salem, Utah

The field on the Davis farm has a medium sandy loam soil, is well drained, and is kept in excellent condition by cultivation and application of barnyard manure. Sugar beets have been grown on it continuously for about 15 years, but the infestation of *Heterodera schachtii* did not appear until about 1919. It is irrigated by the usual furrow method, and the water supply is sufficient to allow an application whenever needed.

Four mononchs were found in this field: *Mononchus papillatus* Bastian, 1866; *M. sigmaturus* Cobb, 1917; *M. macrostoma* Bastian, 1866; and *M. parabrachyurus* Thorne, 1924. Of these *M. parabrachyurus* was not found in 1923, and *M. macrostoma* occurred very rarely.

STUDY OF THE NEMA POPULATION

Soil samples were taken to a depth of 2 feet during 1923, but so few nemas were found in the lowest 6 inches that the following years the samples were taken to a depth of only 18 inches. The soil was collected by the Cobb method, in tubes having an area of one-millionth of an acre. Each sample examined was an aliquot part of from two to six samples, usually two. The soil of each 2-inch depth was col-

lected separately, care being exercised not to mix it with soil from above or below the specified depth.

Samples were collected at intervals which, unfortunately, were not of any definite length of time, as other work interfered. During the summer of 1923 the samples were taken about twice each month, but much of the work had to be abandoned during the autumn and winter. In 1924 it was possible to obtain samples during a longer period, but the sampling was done less frequently. In 1925 samples were collected in every month except January.

The samples were taken from various locations in the fields and in summer were selected at varying distances from the beets, some being taken with many small beet roots in them, while others were collected at considerable distance from the beets.

The temperature of the soil at each 2-inch depth was taken by pushing a rod into the side of the pit made when the tube was dug out. (Tables 1 and 2.[1]) The rod was then pulled out and a thermometer inserted in the hole.

The moisture content of the soil at the various depths was determined by taking 200 grams from each 2-inch sample of soil and drying it 24 hours at 103° C. (Tables 3 and 4.)

TABLE 1.—*Soil temperatures, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	19.0	14.0	28.0	21.0	24.5	27.5	24.5	24.0	26.2
3–4	17.0	13.0	22.0	19.0	22.0	23.0	23.8	23.2	24.1
5–6	16.0	12.0	20.0	17.5	19.5	21.5	23.0	22.3	22.0
7–8	15.0	12.0	18.0	17.0	18.2	21.2	23.0	21.5	21.7
9–10	14.0	11.5	17.0	16.5	17.0	21.0	22.5	20.5	20.9
11–12	13.0	11.5	16.5	16.0	16.0	21.0	22.0	20.0	20.2
13–14	12.5	11.0	15.2	16.0	16.0	20.0	22.0	19.5	20.0
15–16	12.1	10.5	15.0	16.0	15.0	20.0	22.0	19.2	19.9
17–18	11.5	10.0	15.0	16.0	15.0	20.5	21.3	19.1	19.8
19–20	10.5	10.0	14.5	15.8	14.8	20.0	21.1	19.0	19.5
21–22	10.0	9.5	14.5	15.5	14.8	20.0	20.7	19.0	19.2
23–24	10.0	9.0	14.5	15.0	14.5	19.5	20.5	18.8	19.2

1925

Depth (inches)	Feb. 6	Mar. 13	Apr. 18	May 23	June 10	June 29	July 17	Aug. 3	Aug. 22	Sept. 2	Oct. 17	Nov. 21	Dec. 26
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	0.0	1.0	15.0	27.0	19.0	24.0	25.0	25.0	19.0	20.0	6.0	1.0	0.5
3–4	0.0	1.0	13.0	25.0	17.0	23.0	25.0	23.0	18.5	19.5	5.0	1.0	0.8
5–6	0.7	1.2	11.5	21.5	15.0	23.0	25.0	22.5	18.5	19.0	5.0	1.0	1.0
7–8	1.0	1.3	11.0	19.0	15.0	22.7	25.5	22.5	19.0	18.5	5.5	1.5	1.2
9–10	1.0	2.0	10.5	18.5	15.0	22.7	26.0	22.0	19.5	18.5	6.0	2.0	1.5
11–12	1.0	2.2	10.0	18.0	15.0	22.5	26.5	22.0	20.0	18.5	7.0	2.5	2.2
13–14	1.5	2.2	10.0	17.0	15.0	22.0	26.0	22.0	20.0	18.5	8.0	2.7	2.4
15–16	1.5	2.4	9.5	17.0	15.0	22.0	26.0	22.0	20.0	19.0	8.5	3.0	2.6
17–18	1.8	2.5	9.5	16.0	15.0	21.0	25.5	22.0	20.0	19.0	9.0	3.3	2.8

[1] To save space the tables for 1924 are omitted here but are represented on the charts shown on p. 284.

TABLE 2.—*Soil temperatures, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	12.0	13.7	29.0	30.0	28.0	34.0	35.0	30.0	30.0	30.0	12.5
3–4	13.0	13.5	26.0	26.0	25.0	30.0	31.5	28.0	27.0	28.5	9.5
5–6	13.0	13.0	24.0	24.0	24.0	28.0	29.0	25.5	26.5	26.5	7.5
7–8	12.5	13.0	23.0	22.0	22.0	26.0	28.0	24.0	25.5	25.2	6.5
9–10	12.5	13.0	22.0	22.0	21.0	25.0	27.5	23.0	24.5	25.5	6.25
11–12	12.5	13.0	22.0	22.0	21.0	24.0	26.5	23.0	24.0	23.5	6.25
13–14	12.5	13.5	22.0	21.5	21.0	23.0	26.0	22.5	24.0	22.5	7.0
15–16	12.5	14.0	22.0	21.0	21.0	22.0	25.5	22.5	23.8	21.5	7.2
17–18	12.5	14.0	22.0	21.0	21.0	22.5	25.0	21.8	23.8	22.2	8.0
19–20	12.5	14.0	23.0	21.5	22.0	23.0	25.0	21.5	23.5	20.0	8.0
21–22	12.5	14.0	24.0	22.0	22.5	24.0	25.0	22.0	22.5	20.0	8.5
23–24	12.5	14.0	24.0	22.5	23.0	23.0	25.5	22.0	23.5	19.8	9.0

1925

Depth (inches)	Feb. 26	Mar. 20	Apr. 9	May 13	June 24	July 9	Aug. 6	Sept. 10	Oct. 24	Nov. 27	Dec. 18
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
1–2	15.0	17.0	19.0	19.5	24.0	29.0	28.0	15.0	11.0	5.5	0.0
3–4	14.5	15.0	16.0	19.0	22.5	27.0	26.0	14.5	10.5	4.0	0.0
5–6	13.5	11.0	14.0	18.0	22.25	24.0	25.0	14.5	9.5	3.5	0.3
7–8	13.0	9.0	12.0	18.0	22.0	23.0	24.0	14.5	9.0	3.0	1.0
9–10	13.0	8.0	11.0	17.5	22.0	22.0	24.0	15.0	9.0	3.0	1.2
11–12	13.0	7.5	10.0	17.0	22.0	22.0	23.0	16.0	9.0	3.2	1.5
13–14	13.2	7.0	10.0	17.0	22.0	22.0	23.0	16.0	9.5	3.5	1.5
15–16	13.5	6.5	10.0	16.5	22.0	22.0	23.0	17.0	9.6	3.7	2.0
17–18	14.0	6.0	10.0	16.5	22.0	22.0	23.0	17.0	10.0	4.0	2.3

TABLE 3.—*Soil moisture, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1–2	12.0	8.5	10.0	4.0	10.0	4.0	8.0	8.0	2.0
3–4	13.5	13.5	14.0	9.0	12.0	5.5	5.0	8.0	4.0
5–6	14.0	12.5	14.0	11.0	12.0	6.0	5.0	5.5	4.5
7–8	14.0	15.0	16.0	12.0	12.0	6.0	5.5	4.5	5.0
9–10	14.0	15.0	14.5	12.5	14.0	6.5	5.5	5.5	8.0
11–12	13.5	15.0	15.5	13.5	13.5	5.5	5.0	4.5	8.5
13–14	14.5	15.0	15.0	13.0	12.0	5.5	5.0	5.0	9.0
15–16	15.0	20.0	17.0	14.5	13.0	7.0	5.0	6.0	10.0
17–18	17.0	20.0	16.0	14.0	13.0	7.0	5.0	7.0	10.5
19–20	17.0	23.5	14.5	15.0	13.5	7.0	5.5	7.5	11.5
21–22	17.0	23.5	15.5	15.5	15.0	9.0	6.5	8.5	12.5
23–24	16.5	22.5	15.0	15.0	15.0	9.0	7.5	10.5	14.0

1925

Depth (inches)	Feb. 6	Mar. 13	Apr. 18	May 23	June 10	June 29	July 17	Aug. 3	Sept. 2	Oct. 17	Nov 21	Dec. 26
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
1–2	21.0	21.0	15.0	9.5	10.5	6.5	9.5	4.5	8.5	8.0	12.0	14.5
3–4	21.0	18.0	16.0	14.5	13.0	12.0	10.0	9.0	11.5	10.5	11.5	13.5
5–6	21.0	19.0	16.5	14.0	15.0	13.0	11.0	10.5	11.5	11.5	12.0	13.5
7–8	20.5	17.0	16.0	14.0	15.0	13.0	11.5	10.5	12.0	12.5	12.0	13.0
9–10	18.0	17.5	16.0	15.0	15.5	14.5	11.5	11.5	12.5	12.0	11.0	12.5
11–12	18.5	18.0	17.0	14.5	17.5	13.5	12.0	11.0	13.5	12.5	10.5	12.5
13–14	19.0	19.5	18.0	17.0	16.5	14.5	15.5	11.5	14.0	12.5	10.5	12.5
15–16	18.0	20.0	18.0	17.5	17.5	15.5	15.0	13.0	15.5	11.5	11.5	13.0
17–18	17.5	18.5	17.5	16.0	17.5	15.5	14.5	12.5	15.5	11.5	11.5	13.0

TABLE 4.—*Soil moisture, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1–2	10.5	10.5	10.0	10.5	9.5	7.5	5.5	4.5	3.5	9.0	10.5
3–4	14.5	14.0	12.0	13.0	14.0	11.0	11.5	10.5	7.5	11.0	11.0
5–6	15.0	13.5	11.5	15.0	14.5	11.0	11.5	10.5	8.5	11.0	12.0
7–8	16.0	14.5	12.0	14.5	14.0	11.0	11.5	10.5	8.0	11.5	12.0
9–10	14.5	13.5	13.0	15.0	13.0	11.5	11.5	11.0	8.5	11.5	12.5
11–12	15.5	13.0	12.5	15.0	13.0	11.0	11.0	10.5	8.5	11.5	12.0
13–14	15.0	12.0	12.5	14.5	13.0	11.0	10.5	10.0	8.0	12.5	11.5
15–16	14.5	13.0	12.0	13.5	13.0	12.0	11.0	10.0	8.5	12.5	12.0
17–18	15.0	13.5	11.0	13.5	12.5	11.5	11.0	11.0	8.5	13.0	11.5
19–20	15.0	13.5	11.0	12.0	13.0	11.5	11.0	11.5	9.0	12.5	11.5
21–22	15.0	14.0	11.0	12.5	12.5	11.5	11.5	11.5	9.5	12.5	11.0
23–24	14.5	18.5	11.0	11.5	12.5	11.5	11.5	11.5	9.5	12.0	11.0

1925

Depth (inches)	Feb. 26	Mar. 20	Apr. 9	May 13	June 24	July 9	Aug. 6	Sept. 10	Oct. 24	Nov. 27	Dec. 18
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1–2	14.0	9.5	8.5	5.0	5.5	12.0	6.5	11.0	10.0	17.0	15.5
3–4	15.0	10.0	11.0	9.0	10.0	13.0	8.5	11.0	12.5	18.5	16.0
5–6	16.5	14.0	13.0	11.5	12.0	13.5	10.0	12.0	12.5	19.0	15.5
7–8	18.0	16.0	14.5	12.5	13.0	14.5	10.5	11.5	12.0	19.0	15.5
9–10	16.0	17.5	14.0	12.0	13.5	14.5	11.0	12.5	12.5	18.5	14.0
11–12	14.0	14.0	13.5	11.0	12.0	13.5	10.5	12.5	11.5	18.5	14.0
13–14	17.0	13.0	13.0	11.0	11.0	13.5	11.0	13.5	10.5	18.0	15.0
15–16	13.0	14.0	13.0	10.5	11.0	13.0	10.5	13.5	11.0	18.0	15.0
17–18	13.5	13.0	12.5	10.0	11.5	13.0	11.0	13.5	10.5	17.5	15.0

During the summer of 1923 a detailed study was made of all nema species in these fields (Table 5) and the numbers and depths at which they occurred. Observations were made on the life history by noting the stages of growth of the individuals and the times at which the females were found producing eggs.

In 1924 and 1925 this study was limited to *Heterodera schachtii*, the mononchs, and a few species found inhabiting the cysts of *H. schachtii*. It was necessary thus to limit the work because of the great amount of time necessary for making identifications and counts of the individuals found. From observations during the last two years of the experiment and occasional counts, it was found that the data for 1923 were in a large measure typical of those for other years. The species occurring in greatest numbers were usually found in most of the samples, while the more rare forms sometimes occurred in a few samples. Occasionally a single specimen or a small number of a certain species appeared in a single sample and no others were ever found.

Samples in which Rhabditis and some of the Dorylaimus were very numerous were subdivided and an estimate made, based on the count of a small portion. This was never done for mononchs and other less numerous species.

The number of mononchs found and the depths at which they occurred are shown in detail in Tables 6 to 9, inclusive.

TABLE 5.—*Number and species of nemas in field No. 1[a] and in field No. 2[b], 1923*

Species	Field No. 1	Field No. 2
Heterodera schachtii (adults, larvae, and eggs)	101,331	103,602
Rhabditis (mostly larvae of several species)	2,059	5,684
Dorylaimus obtusicaudatus	1,340	1,364
Nygolaimus sp	884	704
Mononchus macrostoma	823	3
Mononchus parabrachyurus	752	
Trichodorus obtusus	505	
Acrobeles butschlii	179	552
Achromadora minima	145	
Cylindrolaimus obtusus	110	31
Tylenchus agricola and T. filiformis	66	262
Tylenchus sp		546
Prismatolaimus stenurus	56	9
Aphelenchus modestus	23	320
Aphelenchus agricola	22	
Dorylaimus regius	18	74
Microlaimus sp	12	
Monhystra sp	12	14
Dorylaimus carteri	10	52
Dorylaimus monhystra	9	
Cephalobus sp	9	
Acrobeles complexus	7	70
Bastiania exilis	6	
Cephalobus nanus	5	
Tylenchus clavicaudatus	4	
Cephalobus subelongatus	3	201
Cephalobus oxyuroides	3	384
Discolaimus texanus	1	
Plectus parietimus	1	26
Plectus sp		58
Mononchus sigmaturus		1,152
Mononchus papillatus		447
Agamermis sp		161
Acrobeles symmetricus		78
Aplectana sp		35
Xiphinema americana		17
Alaimus sp		10
Discolaimus sp		3
Total	108,395	115,859
Mononchs, number of individuals	1,575	1,602
Other free-living species, number of individuals	5,491	10,655
Mononchs, percentage of free-living individuals	22.2	13.1
Heterodera schachtii, per acre population	11,259,000,000	14,800,000,000
Mononchs, per acre population	175,000,000	145,636,000
Other species, per acre population	610,111,000	968,636,000
Total nema population per acre	12,044,111,000	15,914,272,000

[a] On the Bullen farm, near Lewiston, Utah. [b] On the Davis farm, near Salem, Utah.

TABLE 6.—*Number of Mononchus macrostoma, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
1–2		24	2	7		1	4		2
3–4		47	6	73	3		51	3	7
5–6		8	30	128	3	6	87	5	80
7–8		4	20	29	2	6	11	33	29
9–10			3	30	2	4	7	29	1
11–12			2				4	2	2
13–14							1		
15–16	5			2				1	
17–18	6						1		
19–20							1	1	
21–22			3					3	
23–24									
Total	11	83	66	269	10	17	167	77	121

Grand total, 821; population per acre, 91,444,000.

In 1924 only three specimens of *Mononchus macrostoma* were found in field No. 1. Two of these were found October 30, and the other one November 28. On this basis the nema population per acre would be 333,000.

The following numbers of *Mononchus macrostoma* were found in field No. 1 in 1925:

Date	Number	Date	Number
Feb. 6	0	Aug. 3	3
Mar. 13	2	Sept. 2	2
Apr. 18	9	Oct. 17	0
May 23	0	Nov. 21	0
June 10	0	Dec. 26	0
June 29	1		
July 17	1	Total	18

Population per acre, 1,500,000.

TABLE 7.—*Number of Mononchus parabrachyurus, field No. 1*

1923

Depth (inches)	May 16	May 22	June 7	June 16	June 23	July 17	July 25	Aug. 1	Aug. 13
1–2	7	18							
3–4	4	19	2	13	13		4		8
5–6	14	3		20	2	3	14	9	36
7–8	8	2	7	12	9	22	8	39	31
9–10	4		23	11	18	68	31	77	24
11–12			5	1	22	21	8	31	22
13–14			2	2			1	5	5
15–16			4	1				2	16
17–18			4						
19–20			3					1	5
21–22					1			3	2
23–24								1	1
Total	37	42	50	60	65	114	66	168	150

Grand total, 1923, 752; population per acre, 83,555,000.

1924

Depth (inches)	May 22	June 10	June 24	July 7	July 20	Aug. 18	Sept. 24	Oct. 30	Nov. 28
1–2									1
3–4	8	4		2		2	5	1	41
5–6	21		9	3	8	8	22	4	12
7–8	14		13	50	11	16	15	14	12
9–10	3		4	78	49	14	3		24
11–12	1			13	7	3	2	5	7
13–14	2	1	2	7	4				1
15–16	1		3	8					
17–18	1			3					
Total	51	5	31	164	79	43	47	24	98

Grand total, 1924, 542; population per acre, 72,888,000.

TABLE 7.—*Number of Mononchus parabrachyurus, field No. 1*—Continued

1925

Depth (inches)	Feb. 9	Mar. 13	Apr. 18	May 23	June 10	June 29	July 17	Aug. 3	Sept. 2	Oct. 17	Nov. 21	Dec. 26
1–2			1								5	5
3–4	2		15	1			2	1	5	18	6	12
5–6	11		12	5	4	2	2	4	6	9	1	2
7–8	6		5	4	11	4	7	5	4	25		1
9–10	1			7	3	4	5	3	1	24		3
11–12				1	1	2	2	2	1	13		3
13–14						2	2		1	3		
15–16						1	1		2			
17–18												
Total	20		33	18	19	15	21	15	20	92	12	26

Grand total, 1925, 291; population per acre, 24,250,000.

TABLE 8.—*Number of Mononchus sigmaturus, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
1–2		1			2						5
3–4	9	42	19	6	13		41	3	4	4	7
5–6	22	70	21	17	28	7	99	4	10	18	26
7–8		71	27	15	64	3	39	30	10	26	30
9–10	20	107	13	7	19	3	58	8		41	6
11–12		6	4			1	1	5		7	1
13–14	2	24					1			14	
15–16											
17–18		1	1				1				
19–20		3								3	
21–22		2									
23–24											
Total	53	327	85	45	126	14	240	50	24	113	75

Grand total, 1,152; population per acre, 104,727,000.

1924

Depth (inches)	Apr. 6	May 20	June 2	June 17	June 30	July 15	July 28	Aug. 12	Aug. 25	Oct. 3	Nov. 21
1–2											
3–4		3			4	2	4	6			3
5–6	5		1		1	8		8	6	3	1
7–8		2	3		17	4	8	3	5	6	1
9–10	1	2	3			3	4	3	4	2	3
11–12				7		3		3			
13–14				3		2	1	2			
15–16				1			3				
17–18							3				
Total	6	7	7	11	22	22	23	25	15	11	8

Grand total, 157; population per acre, 14,272,000.

TABLE 8.—*Number of Mononchus sigmaturus, field No. 2*—Continued

1925

Depth (inches)	Feb. 26	Mar. 20	Apr. 9	May 13	June 24	July 9	Aug. 6	Sept. 10	Oct. 24	Nov. 27	Dec. 18
1–2											17
3–4	10		1		4	1			2	6	1
5–6	1		5		3	3	2		6	9	5
7–8				6	4	3	3	1	3	9	1
9–10	1	1	1		5	2	1		3	5	24
11–12		4			2	2	1				5
13–14											
15–16											
17–18					1						
Total	12	5	7	6	19	11	7	1	14	29	53

Grand total, 164; population per acre, 14,090,000.

TABLE 9.—*Number of Mononchus papillatus, field No. 2*

1923

Depth (inches)	May 16	May 31	June 11	June 23	June 30	July 12	July 20	Aug. 8	Aug. 29	Sept. 5	Nov. 6
1–2											1
3–4		1	5		2	1	19				4
5–6	1	2		3	22		28			2	
7–8	16	3	4	6	47	4	5				2
9–10	23	12		8	11	1	4			6	
11–12		4	3	8	3	1		6		2	
13–14	45	7	4		5	3		3		13	2
15–16	29	3	5	1	4			1		3	2
17–18	11	3	1		2					6	
19–20	5	4	2		1					4	
21–22	1	2	1								
23–24		2	1							1	
Total	131	43	26	26	97	10	56	10		37	11

Grand total, 447; population per acre, 40,636,000.

In 1924 the following numbers of *Mononchus papillatus* were found in field No. 2:

Date	Number	Date	Number
April 6	1	August 12	0
May 20	6	August 25	1
June 2	3	October 3	0
June 17	0	November 21	1
June 30	0		
July 15	3	Total	15
July 28	0		

Population per acre, 363,000.

Of the 11 soil samples taken from field No. 2 between February 26, and December 18, 1925, only four contained mononchs. Those taken March 20, June 24, July 9, and November 27 contained one each. The population per acre was 363,000.

No *Mononchus parabrachyurus* individuals were found in field No. 2 in 1923.

In field No. 2 the following numbers of *Mononchus parabrachyurus* were found in 1924:

Date	Number	Date	Number
April 6	0	August 12	3
May 20	0	August 25	8
June 2	2	October 3	3
June 17	3	November 21	3
June 30	14		
July 15	4	Total	46
July 28	6		

Population per acre 4,181,000.

In 1925 the distribution of *Mononchus parabrachyurus* in field No. 2 was as follows:

Date	Number	Date	Number
February 26	26	September 10	0
March 20	2	October 24	3
April 9	8	November 27	6
May 23	4	December 18	2
June 24	2		
July 9	3	Total	58
August 9	2		

Population per acre, 5,272,000.

Counting the *Heterodera schachtii* was a complicated process. The larvae and males found free in the soil were counted separately. The numbers of females on the beet roots and those which had fallen off during the washing process were noted. It was also necessary to make an estimate of the eggs and larvae remaining within the brown cysts. A careful count of the eggs in 500 cysts showed that the average number in each was 280, and upon this basis the cyst contents were estimated. During the late autumn and winter months practically all of the cysts containing eggs were full, but with the coming of spring the larvae began to emerge, and from then on during the summer the cysts usually were found partially emptied, except those newly formed on the beet roots. In estimating the contents, the cysts were classed as full, 90 per cent full, 75 per cent full, 50 per cent full, 25 per cent full, 10 per cent full, and empty. When a cyst contained only a few eggs and larvae that could be counted easily, the exact number was taken. There were great numbers of empty cysts, accumulations from former years, which had to be opened in order to count the few eggs or larvae which occasionally remained. Cysts containing nothing but dead eggs or larvae were classed as empty. Most of the cysts from which a portion, or all, of the larvae had emerged were found floating on the surface of the screen residues, while the full ones were collected in the heavier screenings.

The *Heterodera schachtii* population per acre of field No. 1 was 11,250,000,000 in 1923, 2,251,000,000 in 1924, and 34,821,000,000 in 1925. Data for field No. 1 were incomplete. The *H. schachtii* population per acre of field No. 2 is shown in Table 10.

TABLE 10.—*Number of Heterodera schachtii, field No. 2*

1923 [a]

Depth (inches)	June 23	June 30	July 12	July 20	Aug. 8	Sept. 5	Nov. 6
1–2	490	1,260	31,500	610	735	212	162
3–4	280	1,148	10,366	5,611	3,154	1,114	
5–6	140	100	10,145	3,395	436	1,000	408
7–8			6,143	486	1,447	249	375
9–10		3,696	2,957	1,536	2,153	411	154
11–12		2,156	1,795	735	1,431	386	220
13–14		210			611	290	470
15–16						1,172	986
17–18				201		150	184
19–20		70				287	168
21–22						100	94
23–24							
Total	[b]910	8,640	62,906	12,574	9,967	5,380	3,221

Grand total, 103,598; population, per acre, 14,800,000,000.

1924

Depth (inches)	June 2	June 17	June 30	July 15	July 28	Aug. 12	Aug. 25	Oct. 10	Nov. 25
1–2	288	53	68	241	377	920	908	26	109
3–4	98	56	1,443	1,355	180	1,845	3,041	98	1,231
5–6	462	252	1,414	837	413	675	2,853	420	2,457
7–8	116	71	2,472	598	849	1,437	1,470	127	1,649
9–10	694	241	1,093	1,879	186	810	841	81	3,466
11–12		75	1,360	210	198	398	517	8	1,701
13–14		14	28	88	664	223	300	81	6,003
15–16		32	15	75	1,590	326	945		30
17–18		21			1,278		1,071		383
Total	1,658	815	7,893	5,283	5,735	6,634	11,946	841	17,029

Grand total, 57,834; population per acre, 6,436,000,000.

1925

Depth	Feb. 26	Mar. 20	Apr. 9	May 13	June 15	June 24	July 9	July 21	Aug. 6	Aug. 15
1–2	4,589	3,312			410	2,313	2,811		2,544	1,809
3–4	624	1,554	500		1,500	2,438	3,811	65	2,024	1,767
5–6	530	2,604	175	49	1,175	693	2,704	1,260	2,935	230
7–8	298	328	32	423	1,609	1,191	1,396	3,486	1,687	28
9–10	362	1,278	2,000	62	398	668	1,577	724	4,904	275
11–12	5,471	1,245	3,486	847	944	578	2,430	66	4,303	637
13–14	949	20		826	4,838	1,723	3,445	1,423	5,653	2,625
15–16	6,698	68	108	290	3,683	175	710		772	1,427
17–18	8,123	250	175		598		352	25	350	1,860
Total	27,644	10,659	6,476	2,497	15,155	9,779	19,236	7,049	25,172	10,658

Grand total, 134,325; population per acre, 13,444,000,000.

[a] No record was available of the samples taken May 16, May 31, June 11, and Aug. 29.
[b] Incomplete.

In 1924 the populations of *Heterodera schachtii* of both fields were much lower than in 1923 or 1925. There are two reasons for this: (1) The year 1923 was, in general, a normal year for successful sugar-beet production, and many of the infested beets made a good growth. This meant an abundance of roots, upon which developed large populations of *H. schachtii*. However, 1924 was an exceedingly poor year for sugar-beet growth and the young beets found difficulty in becoming established. (2) The large numbers of *H. schachtii* hatching in the spring of 1924 attacked these weak seedlings

and killed practically all of those in the infested areas. This resulted in the destruction of a large percentage of the *H. schachtii* which had hatched and entered the beet roots, and only comparatively small numbers survived on the few beets remaining.

The growing season of 1925 was excellent, and the young beets were well established early in the spring. Even on the most severely infested areas many of them made a fair growth because the *Heterodera schachtii* populations were low, as the previous year had been an unfavorable one. Also the fertility of the soil was higher because on the infested areas few beets had been produced in 1924 and the soil had been kept fallow by cultivation. For these reasons, the beets grew thriftly, developing large root systems upon which the *H. schachtii* increased to such enormous numbers that by the end of 1925 the populations had again reached a high point.

It may safely be predicted that these high populations will destroy practically all of the young beets in 1926 and that by the end of the year the populations will be much lower than at the beginning.

No attempt has been made to place the *Heterodera schachtii* populations on the graphs (figs. 3 and 4) because the bulk of the population occurs in the cysts and these are moved about in the soil by plowing, cultivating, and other cultural operations until their locations have no relation to the actual point of habitat.

LIFE HISTORY OF MONONCHS IN THE FIELD

Definite broods or cycles of mononchs were not observed in any of the species studied. Every collection contained individuals of various sizes, and examination of the denticles in the pharynx showed that the smaller forms had yet to molt one or more times. Cobb[4] states that mononchs probably molt four times, and all of the observations made on the specimens collected in this experiment bear out this statement, although like Steiner and Heinly[5], the writer did not observe specimens making a first molt just after emerging from the eggs. No method was devised for learning how long the eggs lie in the soil before the larvae hatch or the rate of growth of the young mononchs. From the fact that very small individuals were found three months after the last ova-bearing females were seen, it would appear that either some of the eggs do not hatch immediately or that growth is very slow.

Temperature is apparently an important factor in egg production. In field No. 1 no gravid females of *Mononchus parabrachyurus* were found in the winter of 1924–25 during the months of October, November, February, and March. In field No. 2 gravid females were collected in October and February but none in November and March. Tables 3 and 4 show that the temperatures of both fields fell to about 10° C. or lower on the dates when gravid females were not found.

In field No. 2, *Mononchus sigmaturus* were found gravid on November 27, 1925, when the temperature was below 5° C. In January, 1926, they were found gravid when the temperature was 2.5°. This

[4] COBB, N. A. THE MONONCHS. (MONONCHUS BASTIAN 1866.) A GENUS OF FREE-LIVING PREDATORY NEMATODES. Soil Sci. 3: 431–486, illus. 1917.

[5] STEINER, G., and HEINLY, H. THE POSSIBILITY OF CONTROL OF HETERODERA RADICICOLA AND OTHER PLANT-INJURIOUS NEMAS BY MEANS OF PREDATORY NEMAS, ESPECIALLY BY MONONCHUS PAPILLATUS BASTIAN. Jour. Wash. Acad. Sci. 12: 367–386, illus. 1922.

indicates that they reproduce at much lower temperature than do *M. parabrachyurus*. No gravid females were found on December 18, 1925, when the soil was frozen to a depth of 8 inches. After a box of the soil had remained in the laboratory at ordinary room temperatures until December 23, gravid females were numerous. This would indicate that there is prompt response to a rise in temperature at that time of year.

On August 8, 1923, about 50 pounds of soil from field No. 2 was placed in a cool cellar. At that time a count showed 12 *Mononchus sigmaturus* per pound. On December 6, they had increased to 308 per pound, but on March 8, 1924, only a few were to be found. Nothing was determined as to the cause of their disappearance.

Cessation of egg production during the summer months is perhaps an adaptation to the arid conditions which prevailed in these soils before the advent of irrigation, but is more probably caused by the high temperature of the soil at this time. That such is the case seems to be borne out by the fact that these same species of mononchs collected from the cool, moist soils along canyon and valley streams often are found producing eggs during the summer months. This may be due to the absence there of the high temperatures common in the valley fields or perhaps to the development of biologically adapted strains of the same species. No transfers of mononch colonies have been made to determine this point.

No gravid females were found among the 823 specimens of *Mononchus macrostoma* collected in 1923. Many were adults, but it was evident that in this field the period from May 16 to August 13 was not the time of egg production. Specimens collected in other fields during the months from November to March contained eggs, except those collected during very cold periods. Immature specimens are almost always present in collections made at any time of year. Specimens from stream banks and springs often are gravid during the summer months.

Considerable numbers of gravid females of *Mononchus parabrachyurus* were found in October, and occasional specimens occurred during the winter months when the temperature was above 10° C. However, the greater number appeared in the April and May collections, and this is probably the time of maximum egg production.

Gravid females of *Mononchus papillatus* were numerous in the April and May collections, but none were found later. Immature specimens were numerous during the summer months, again pointing to a delayed hatching or slow growth of the larvae. Two gravid specimens were found in September, 1923, but none in November.

In rare instances gravid females of *Mononchus sigmaturus* have been collected in July and August, but the greatest numbers appeared in the March, April, and May collections. Egg production apparently ceases at about 2.5° C.

CAUSE OF REDUCTION IN MONONCH POPULATION

The most striking fact shown by the data on mononch population (Tables 6 to 9, and text lists on p. 270) is the reduction in the numbers of all species, with the one exception of *Mononchus parabrachyurus* in field No. 2, where this species was first found in 1924.

Mononchus macrostoma, so abundant in field No. 1 in 1923, apparently was just as numerous in 1922 when the colony was discovered. Between August 13, 1923, and May 22, 1924, they practically disappeared from the field, as is shown in Table 3. The cause is not definitely known, but in July and August, 1923, several specimens were found that were heavily parasitized by elongate-ellipsoidal sporozoans. In some instances the body cavities were almost completely filled, especially about the region of the reproductive organs. Unfortunately, these specimens were lost and a definite description of the parasites can not be given. Similar parasitized specimens have not been collected from other localities.

Specimens of *Mononchus parabrachyurus* have frequently been found parasitized by sporozoans (fig. 1). In rare instances sporozoans apparently cause the death of the mononch. The sporozoans usually congregate in large numbers about the reproductive organs (fig. 1), and such infested specimens have not been found producing eggs. In February, 1926, 30 per cent of the mononchs in field No. 1 were parasitized, and there was little doubt that these sporozoans were the cause of the reduction in numbers. A few specimens contained rodlike bacteria in addition to the sporozoans (fig. 2).

Sometime after specimens had been collected from field No. 1 about 200 specimens were collected from fields near it, but in none of them were parasitized specimens found. The attack was therefore confined to that one field and was not epidemic to the whole section.

Mononchus sigmaturus has also shown a steady reduction in numbers, but no parasites ever have been observed in any of the specimens examined from field No. 2 or other neighboring fields. Dead specimens frequently have been found, but nothing could be seen in the bodies that would indicate the cause of death. A specimen collected at Ogden, Utah, was heavily infested with a sporozoan similar to, perhaps identical, with one found in *M. parabrachyurus*.

Mononchus papillatus almost disappeared from field No. 2 during the winter of 1923–24, but nothing is known of the cause. This species is less frequently found inhabiting cultivated fields, and observations on it have been limited.

FOOD HABITS OF MONONCHS

Mononchus macrostoma has been observed feeding on nemas, rotifers, and other microscopic soil organisms. The bulk of its food appears to be organisms other than nemas. Of the nemas devoured, the majority were Rhabditis, and only three were *Heterodera schachtii*—two larvae and one male. Rhabditis apparently is preferred because the cuticle is thin and the body contents easily drawn out. The cuticle usually is discarded, but occasionally it is swallowed.

Mononchus parabrachyurus eats as its usual food rotifers, protozoans, and other microorganisms. Nemas rarely are devoured, the only records obtained being one Rhabditis and one *Heterodera schachtii* larvae. The cuticle of both specimens was discarded. It was impossible to get many of these mononchs to attack other nemas when they were kept constantly about them for as long as two weeks at a time. They apparently were able to obtain minute organisms from the soil particles and water in which they were kept. Sometimes they would attack other nemas, but after a brief struggle would

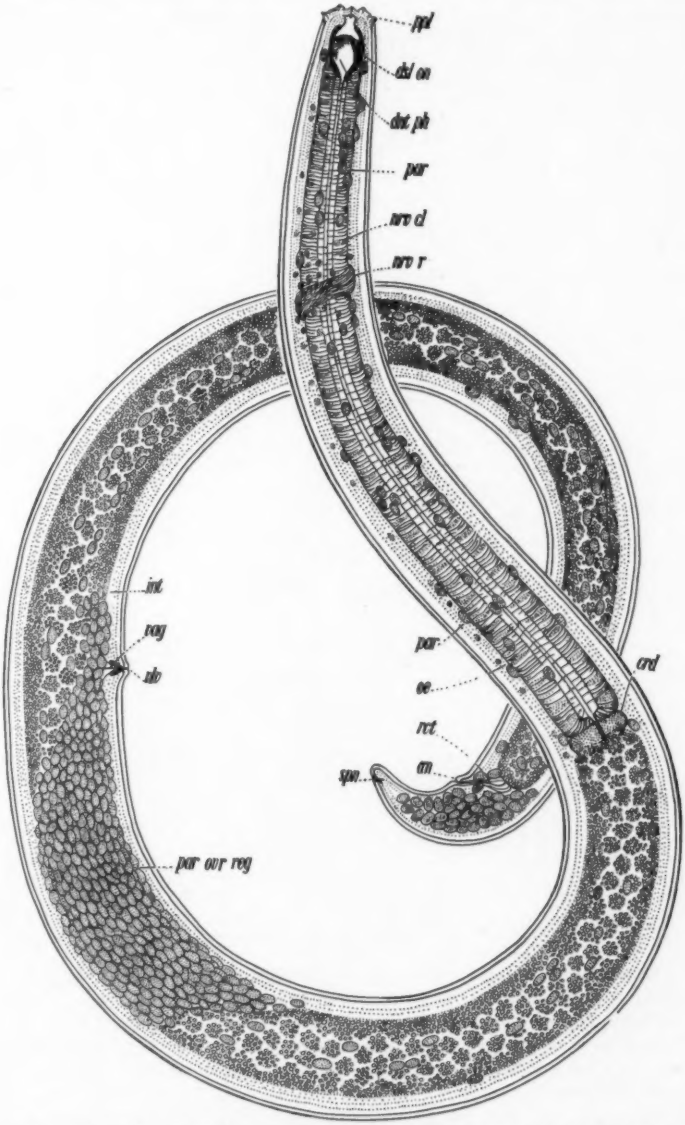

FIG. 1.—*Mononchus parabrachyurus* infested with sporozoan parasites which have reached all parts of the body, congregating especially about the region containing the reproductive organs. Such large colonies sterilize the mononch and prevent the production of eggs

Key.—*an*, anus; *crd*, cardia; *dnt ph*, denticles of pharynx; *dsl on*, dorsal tooth; *int*, intestine; *nrv cl*, nerve cell; *nrv r*, nerve ring; *oe*, esophagus; *par*, parasite sporozoan; *par ovr reg*, parasite in ovary region; *ppl*, papillae; *rct*, rectum; *spn*, spinneret; *vag*, vagina; *vlv*, vulva.

release them. They appeared to be handicapped in not having firm soil particles on which to anchor themselves by the spinneret. Under field conditions they may more successfully attack and devour nemas, but from laboratory observations it appears that they prefer other forms of prey.

Mononchus sigmaturus, like *M. parabrachyurus*, appears to prefer microorganisms other than nemas for food. It frequently attacked the eggs of *Heterodera schachtii* when the cyst had been broken open, and sometimes it was able to puncture both the eggshell and cuticle of the larvae and draw out the body contents (fig. 5). Small nemas were attacked occasionally but often were released without having been punctured or killed.

FIG. 2.—Photomicrograph of neck of *Mononchus parabrachyurus*, showing sporozoan parasites and rodlike bacteria in the body cavity

Mononchus papillatus was found by Steiner and Heinly,[6] to be especially voracious and to attack other nemas readily. Several times during the summer of 1923, when the screen residues from the soil samples were examined, these mononchs were found devouring other nemas. Even the large males of *Heterodera schachtii* are readily attacked and the body contents drawn out (fig. 4). It is not unusual to find partly devoured nemas in soil where these mononchs are numerous, perhaps bearing out the findings of Steiner and Heinly that they kill for pleasure when their appetites have

[6] STEINER, G., and HEINLY, H. THE POSSIBILITY OF CONTROL OF HETERODERA RADICICOLA AND OTHER PLANT-INJURIOUS NEMAS BY MEANS OF PREDATORY NEMAS, ESPECIALLY BY MONONCHUS PAPILLATUS BASTIAN. Jour. Wash. Acad. Sci. 12: 367-386, illus. 1922.

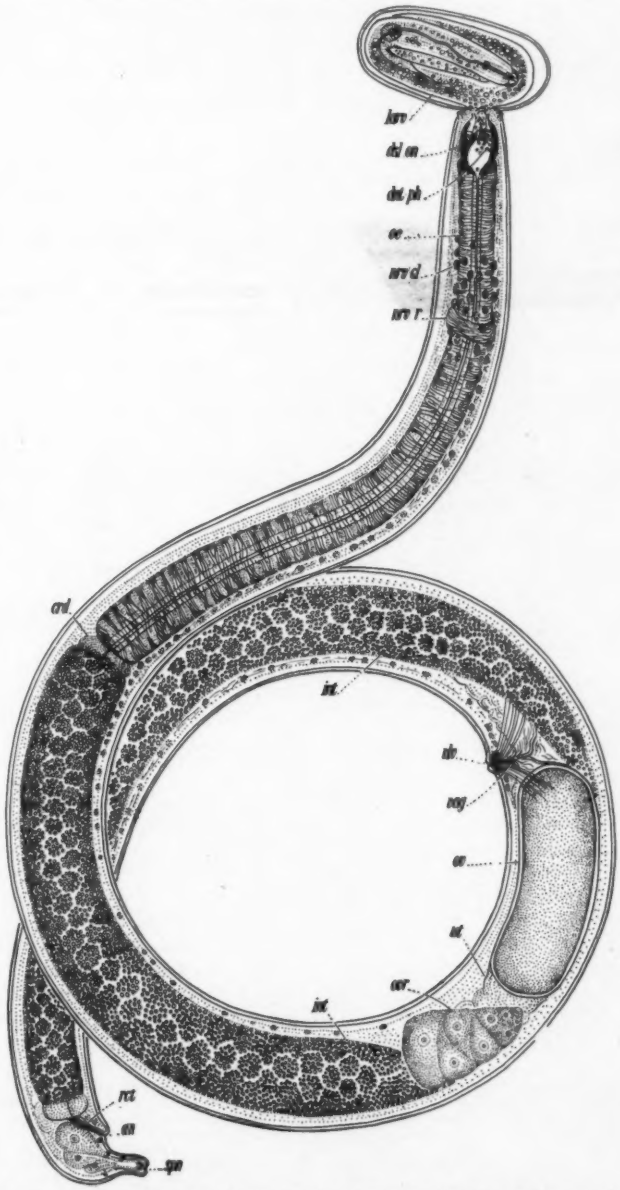

FIG. 3.—*Mononchus sigmaturus* devouring an egg of *Heterodera schachtii*. The larva (larv) can be seen coiled in the egg. The mononch has punctured both the egg and the cuticle of the larva and is drawing out the granular contents of the body

Key.—*an*, anus; *crd*, cardia; *dnt ph*, denticles of pharynx; *dsl on*, dorsal tooth; *int*, intestine; *larv*, larvae; *Heterodera schachtii*; *nrv cl*, nerve cell; *nrv r*, nerve ring; *oe*, esophagus; *ovr*, ovary; *ov*, ovum or egg; *rct*, rectum; *spn*, spinneret; *ut*, uterus; *vag*, vagina; *vlv*, vulva.

been satisfied. Immature females of *H. schachtii* attached to beet rootlets were fed to these mononchs, and on two occasions a mononch was observed attacking one of them and drawing out the body contents.

ECONOMIC IMPORTANCE OF MONONCHS

From the present evidence it appears that the native mononchs of the sugar-beet fields of Utah are subject to the attacks of sporozoans and other parasites or diseases that frequently destroy almost the entire colony. Under such conditions there will be no opportunity for the mononchs to become of economic importance. It might be possible, however, to transfer uninfected individuals from other localities and establish colonies of species unknown in a locality, so that they might escape the destructive parasites and diseases. But such procedure would be attended by many difficulties.[7]

FIG. 4.—Photomicrograph of head of *Mononchus papillatus* devouring a small male *Heterodera schachtii*. The body contents are being sucked out, but the cuticle is discarded

The food habits of *Mononchus sigmaturus* and *M. parabrachyurus* indicate that they have but little, if any, economic importance. *M. macrostoma* is also of doubtful value.

Mononchus papillatus would doubtless be of considerable aid in the control of *Heterodera schachtii* if some method could be devised for maintaining large colonies in the infested fields.

The greatest increase in the number of mononchs occurs in April and May when other free-living forms also are exceedingly numerous. At this time there are many *Heterodera schachtii* larvae moving from the cysts to the sugar beets and some of them are destroyed by the mononchs. However, the mononchs seem to prefer the various species of Rhabditis, which are even more abundant than *H. schachtii* at this time of year. The enormous populations of *H. schachtii* that develop during the summer would not have a stimulating effect on the mononch population because reproduction does not occur at that time.

[7] COBB, N. A. TRANSFERENCE OF NEMATODES (MONONCHS) FROM PLACE TO PLACE FOR ECONOMIC PURPOSES. Science (n. s.) 51: 640-641. 1920.

IMPORTANCE OF MOISTURE

Water is the most vital factor in nemic activity. A film about the soil particles is essential for movement and the continuation of life processes. Without it nemas must enter a quiescent form in which they lie dormant until its return.

How long this dormant condition may continue without death to the nema probably varies with the different species, but it is certain that it is normal for those here discussed to remain in this condition during the hot dry summer months. That such is the case has been determined definitely, for practically all the species here listed, by examining specimens in soil taken from unirrigated fields during the summer months.

During this quiescent stage the mouth apparently is closed by a sort of plug, the nema is inert, and respiration practically ceases. Response to moisture is almost immediate, and it is only by working rapidly that one can wash and examine a dry soil sample before the nemas begin to show signs of life. The mononchs usually revived in from 10 to 20 minutes.

It seems peculiar that some species prefer to remain in the first few inches of soil where drying out is frequent. Other species live deeper in the soil, but often some individuals of these species will be found quiescent in the hot dry soil when at a depth of 6 or 8 inches there is ample moisture, indicating that they sometimes apparently make no effort to avoid passing into this quiescent condition.

In the two fields under study nemic activity ceased when the soil-moisture content dropped to 7 or 8 per cent. This figure no doubt would vary slightly for soils of different textures. The mean moisture and temperature points for these fields are indicated in Figure 5.

The data given in Tables 6 to 9, inclusive, show that mononchs generally avoided the upper 2 inches of soil except when the moisture content was high and the temperature low. For example, in field No. 1, on May 22, 1923, both *Mononchus macrostoma* and *M. parabrachyurus* were gathered near the surface after several days of cool weather and showers.

Figure 6 shows that in field No. 1 there was a general tendency for *Mononchus parabrachyurus* and *M. macrostoma* to go deeper into the soil as the moisture decreased. However, such migration also occurred when there was an increase in temperature. As is brought out later in this paper, temperature may be the more important factor.

Field No. 2 (fig. 6) had a more erratic moisture content, owing to the fact that irrigation water was applied at various intervals. There apparently is no correspondence between the average depth of habitat of *Mononchus sigmaturus* and *M. papillatus* and the moisture content of the soil.

In Figure 6 the moisture line was obtained by charting the actual percentage of moisture at its average point. The temperature line was obtained in like manner. The habitat lines for the mononchs were obtained by the same formula and represent the average points of habitat of the various species.

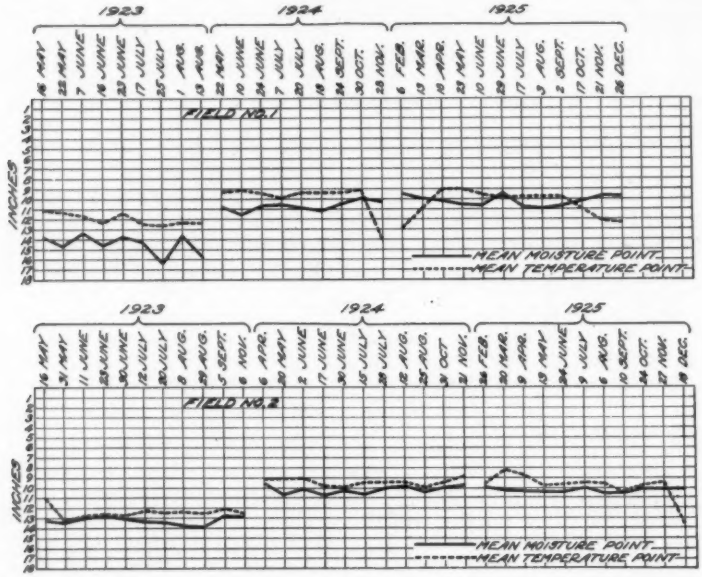

FIG. 5.—Mean moisture point and mean temperature point of fields Nos. 1 and 2, 1923, 1924, and 1925

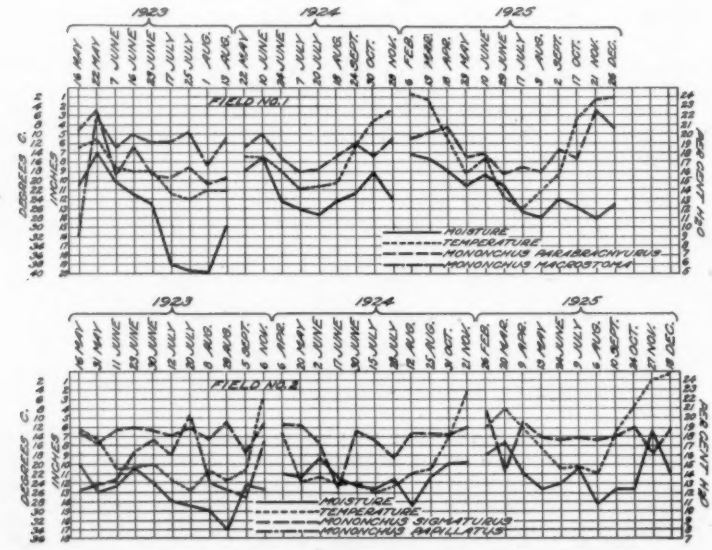

FIG. 6.—Moisture, temperature, and average points of habitat of *Mononchus parabrachyurus* and *M. macrostoma* in field No. 1, and of *M. sigmaturus* and *M. papillatus* in field No. 2, 1923, 1924, and 1925

It may be concluded from Figure 6 that mononchs are but little influenced by moisture in selecting a habitat, provided the soil contains more than the 7 or 8 per cent of moisture necessary for their activity. They do exhibit a marked tendency to avoid the upper 2 inches of soil, in which frequent drying occurs.

IMPORTANCE OF TEMPERATURE

Figure 6 shows that temperature has little influence on the point of habitat of any mononchs except *Mononchus parabrachyurus*. The occurrence of this species in field No. 1 seemed to depend largely on the temperature.

In field No. 2 the average point of habitat of *Mononchus sigmaturus* was very close to 7 inches in depth at almost all times of the year. How can the great variations in the depth at which they occurred on June 17, 1924, and March 20, 1925, be explained? Since moisture and temperature were not the cause of these variations, it seems possible that some organism preferred for food may have been present at the lower depths, causing the mononchs to migrate down to those points.

The wide variation in average point of habitat of *Mononchus macrostoma* on May 16, 1922, also seems mysterious. It seems peculiar that this species, which usually prefers the cool, moist soils of stream banks, should have lived so close to the surface in field No. 1 during the extremely hot dry summer of 1923. Observations on this species made in other fields rarely have shown that this species prefers habitats so deep as those in which they were found on May 16, 1923. Usually they are found at an average depth of from 6 to 12 inches.

The erratic average point of habitat for *Mononchus papillatus* in 1923, field No. 2 (fig. 6), seems quite typical of this exceedingly active species. It is probable from observations in this and two other fields that these mononchs migrate considerably in their search of food, without any regard whatever for temperature or moisture. However, the bulk of the specimens are almost invariably collected at a depth of 6 to 12 inches.

SUMMARY

The nemas of the genus Mononchus inhabiting the sugar-beet fields of Utah and southern Idaho are chiefly of four species: *Mononchus papillatus* Bastian, *M. macrostoma* Bastian, *M. sigmaturus* Cobb, and *M. parabrachyurus* Thorne. These species often occur in sugar-beet fields infested with the destructive sugar beet nematode, *Heterodera schachtii* Schmidt. They prefer the lighter sandy soils where they are sometimes found in populations as high as 300,000,000 per acre. In the heavy soils they occur much less frequently.

A study was made of the populations in two of these fields with the primary purpose of determining if the mononchs were of economic importance in the control of *Heterodera schachtii*. The general habits of mononchs in relation to temperature, moisture, food supply, and diseases or enemies were also studied.

The principal period of mononch reproduction occurs during the months of March, April, and May and there is probably but one generation each year. The populations vary greatly from year to year. *Mononchus macrostoma* and *M. parabrachyurus* are attacked by Sporozoan parasites that sterilize them and reduce their numbers. Other undetermined causes greatly reduced the numbers of *M. sigmaturus* and in one field practically exterminated *M. papillatus*.

Mononchus papillatus was found to be the most voracious species and frequently was observed devouring the larvae and males of *H. schachtii*. The other three species appeared to prefer rotifers and other microorganisms as food.

Because of their unstable populations, time of reproduction, and food habits it is doubtful if the mononchs studied were of economic importance in the control of the sugar-beet nematode.

Nemic activity ceased when the soil moisture content dropped to 7 or 8 per cent in the two fields under study. Mononchs are able to lie dormant through the dry summer months but revive in 10 to 20 minutes when water is applied to the soil.

Mononchus parabrachyurus appeared to migrate deeper into the soil during the hot summer months but the other species studied apparently were not influenced by temperature.

TWO WATER MOLDS CAUSING TOMATO ROOTLET INJURY[1]

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INTRODUCTION

During May, 1926, the writer isolated some possibly parasitic fungi from affected rootlets of tomato (*Lycopersicum esculentum* Mill.) seedlings in greenhouses of the United States Department of Agriculture in the District of Columbia and at the Arlington Experiment Farm, Rosslyn, Va. The underground portions of these seedlings were, on the whole, in very good condition, injury being confined usually to a relatively small portion of the more minute rootlets, which exhibited yellowish or brownish discoloration at the tip and for several millimeters to a centimeter upward.

This material while scarcely in a condition to cause practical concern, yielded nevertheless a numerous array of fungous cultures referable to more than a dozen species, most of them to a certain degree parasitic. The genus Pythium was very liberally represented in this collection, as, indeed, might be expected from the very usual association of many of its members with rootlet-tip injury in numerous representatives of the higher plants. The mycelial form identified as *Rhizoctonia solani* Kühn (*Corticium vagum* B. & C.) made its appearance in scores of cases. A less predictable feature of these isolations was a frequent recurrence of two water molds, which, because of the paucity of information as to the association of members of the Saprolegniaceae with root injury in general, deserve further discussion.

OCCURRENCE OF APHANOMYCES EUTEICHES

One of the water molds which appeared more than a dozen times was, in the absence of cross inoculation experiments, provisionally identified as *Aphanomyces euteiches* Drech., a fungus known as the cause of a very destructive type of root rot of peas prevailing especially during seasons of excessive rainfall (*4*).[2] A comparison of the strains isolated from tomato roots with others derived from pea roots showed general agreement in morphological detail, including the very distinctive peculiarity presented in the sinuous inner contour of the thick oogonial wall. Mature oospores of the fungus were recognized in the tissues of a number of affected rootlet tips. With a few exceptions the rootlets that yielded *A. euteiches* yielded no other fungus. Appearances indicated actual though not severe pathogenicity—a degree of pathogenicity important not so much on account of any potential damage to tomatoes as on account of the increased opportunity afforded the parasite for

[1] Received for publication Sept. 25, 1926; issued March, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 292.

maintaining itself until such time as an ensuing crop of peas becomes available. In regions where tomatoes and peas are cultivated on a example, in some sections of Maryland, the fungus would thus be sufficiently extensive scale to play important parts in a rotation, as for better able to survive from season to season in dangerous quantity.

PLECTOSPIRA MYRIANDRA N. G.; N. SP.

OCCURRENCE

The second water mold, *Plectospira myriandra*, was encountered about as frequently as the first, but only on potted tomato seedlings from the greenhouses at Arlington farm. Its relationship to the host appeared to be quite similar, injury being limited to brownish discoloration and eventual death of occasional rootlet tips. The damage to the material examined was rather slight. Growths originally obtained on corn-meal agar plates were freed of contaminating bacteria by the method described by Brown (*1*), which consists in making transfers from the under side of thick, plain-agar plates. Thus purified, the fungus was subsequently cultivated on artificial media like corn-meal agar, Lima-bean agar, Lima-bean decoction, and pea decoction. Advantage was also taken of the well-known method of inducing zoospore formation by the transfer of vigorous mycelia to sterile water.

MORPHOLOGY

On corn-meal agar the fungus produces a colorless growth devoid of aerial mycelium, having much the general appearance of *Aphanomyces euteiches* or *A. helicoides* Minden (*7, p. 555–562*), and exhibiting, like these, a well-defined radial effect. Under the microscope the mycelium is seen to be composed of hyphae of low refringency, branching at moderate intervals. As in the species of Aphanomyces mentioned, each hyphal element maintains approximately the same diameter from its origin to its termination. The more delicate branches, such as are produced, for example, when portions of mycelium are transferred to water (fig. 1, A) and including also the filaments bearing the antheridia, not infrequently measure less than 2μ in diameter—probably as slender mycelial elements as are known in the Saprolegniaceae.

Zoosporangia are obtained when fresh growth from liquid or agar media is transferred to water, corn-meal agar seemingly providing the most satisfactory substratum that has been used for their production. Under suitable conditions massive complexes are formed, composed of folded, inflated elements usually twice and not rarely three times the diameter of the largest vegetative hyphae and bearing short digitate branches—the whole a compact, intricate apparatus. The basal protions of Figure 1, B and D, represent two such structures of only moderate proportions. The larger examples, which measure more than 200μ in diameter, have a bulk five or ten times as great and are very easily seen with the naked eye. In Figure 1, C and F, are shown still simpler types, while the very simplest, consisting, indeed, of only a single inflated element, is represented in Figure 1, E. These complexes are apparently always borne terminally on a hypha of ordinary diameter, from which, on attaining definitive size,

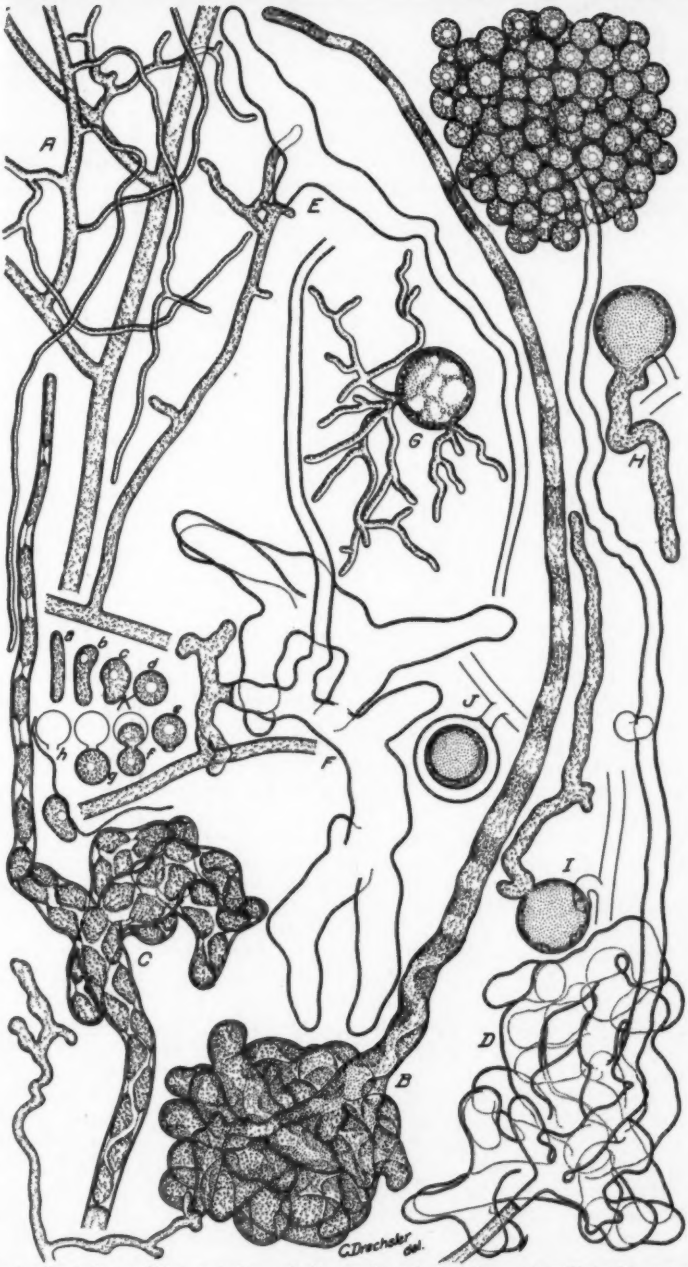

FIG. 1.—Vegetative condition, asexual reproduction, and germination of oospore of *Plectospira myriandra* as occurring in material grown in pure culture on corn-meal agar and transferred to sterile water. All figures drawn with aid of camera lucida, × 500. A, mycelium, the larger hyphae submerged in substratum, the very delicate filaments growing free into surrounding water; B, sporangium showing contents becoming separated into zoospore initials; C, sporangium of small size with zoospores ready for discharge; D, sporangium after evacuation with irregular mass of encysted zoospores at mouth of efferent element; E and F, two small sporangia after discharge; G, oospore in water germinating by delicate hyphae; H and I, oospore embedded in substratum germinating by single stout germ tubes; J, disappearance of oospore wall initiating process of germination; K, *a* to *h*, successive stages in rounding up and an encyst ment of zoospore followed by production of papilla, evacuation of contents ,and escape in motile form

they are regularly set off by a fairly thick septum. As their enlargement nears completion, there is produced distally from one of the distended parts—often from one that appears as somewhat of a direct prolongation of the supporting hypha—a filamentous element of diminishing diameter that attains finally a length varying from 100μ to 600μ or 700μ. This filamentous element, which is sometimes marked by abrupt bends at irregular intervals or again may be of regular outline throughout its course, serves in the evacuation of the sporangium as an efferent tube.

Zoospore formation is associated, as in related forms, with protoplasmic changes (fig. 1, B), which in the efferent hypha differs in no particular from that characteristic of the genus Aphanomyces and results here also in a single series of protoplasts connected by delicate strands (fig. 1, C). Within the inflated elements the process results usually in two parallel series of such protoplasts. When the tip of the efferent filament gives way evacuation takes place, often with violent rapidity, at an approximate rate of perhaps 10 zoospores a second, so that scores of these bodies may be observed at the mouth of the tube in their original cylindrical shape, or in scarcely altered early stages of contraction. The total number of zoospores delivered from a single efferent element varies from a minimum of about 20 to 25 to a usual maximum of from 400 to 500, depending on the size of the sporangium involved. The sporangial complexes that exceed at all considerably the proportions of those shown in Figure 1, B and D, usually are provided with plural efferent tubes, each of which functions independently in evacuating portions of the aggregate apparatus, which may thus best be regarded as constituting a compound structure made up of the basal parts of a number of sporangia.

The zoospores remain massed at the mouth of the sporangium in an encysted condition for a period of approximately two hours (fig. 1, D), after which they escape from the cyst wall (fig. 1, K), swim about actively for a time, round up a second time, and under suitable conditions, germinate. The details associated with the discharge of the cyst contents are identical with those described for *Aphanomyces euteiches*, even with respect to the dimensions of the papilla and of the short evacuation tube that persists on the empty cyst wall.

The sexual stage of the fungus develops readily on various media, being produced in quantity, for example, on corn-meal agar. The oogonia appears as subspherical bodies, occasionally intercalary or laterally intercalary in position (fig. 2, D and E) but much more frequently formed terminally on relatively short branches from the stouter hyphae (fig. 2, A–C, F–I). These branches do not generally exceed in length the diameter of the oogonium and frequently measure considerably less. The septum delimiting the oogonium may be inserted nearly tangent to the subspherical part, but more typically is inserted somewhat lower, including, as it were, a portion of the stalk. In the mature parthenogenetic apparatus the oogonial wall often seems lightly sculptured internally, and at times it presents the appearance of numbers of minute, scattered pits. Whether these inconspicuous features are to be homologized with the pronounced pits distinctive of the oogonia of various genera of Saprolegniaceae is not altogether clear, but would seem very doubtful.

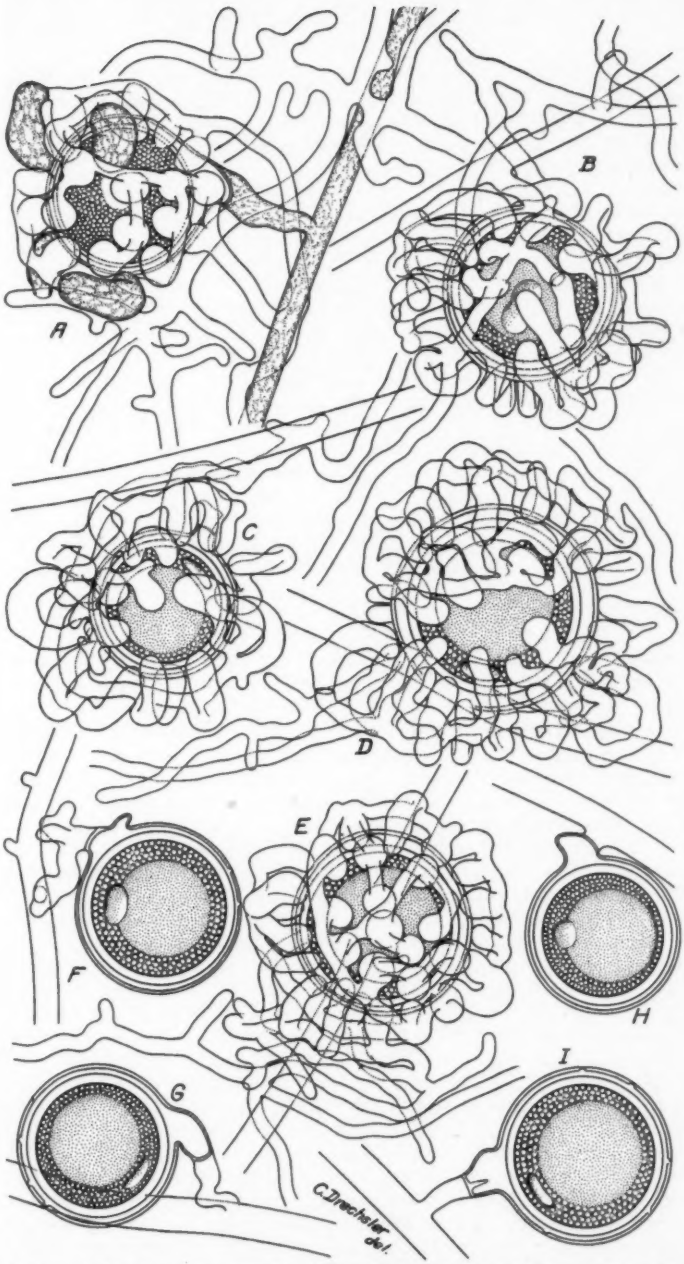

FIG. 2.—Sexual and parthenogenetic apparatus of *Plectospira myriandra* drawn from corn-meal agar cultures with aid of camera lucida, × 1,000. A to E, oogonia invested with numerous antheridial elements, terminal on short branches in A, B, and C; laterally intercalary in D and E (the four antheridia in A with contents shown by stippling are of potentially functional type); F to I, mature parthenogenetic oogonia with oospores

In most cultures from one-half to two-thirds of the eggs are usually produced parthenogenetically. (Fig. 2, F–I.) The remainder are formed in the presence of antheridial elements in extraordinary abundance. (Fig. 2, A–E.) The elements are uniformly of declinous origin, arising from delicate, sinuous hyphae. In the proximity of the oogonium one or several of these hyphae put out branches, the resultant filaments enveloping different regions of the female structure and giving off at short intervals, either directly or on short, spurlike branches, elements having the shape and the positional and contact relations usual for antheridia. Most of these elements never are delimited by septa and soon appear as empty structures in consequence either of the withdrawal or the degeneration of their contents. In most instances, however, several of the structures, usually terminal in position and often distinguished from the others by greater size, a thicker wall, and more extensive contact with the oogonium, will be found delimited by a septum and filled with dense protoplasmic material. (Fig. 2, A.) These structures have all the appearance of true antheridia. In more advanced material they are, like the rudimentary elements, devoid of contents.

Owing to the difficulty of accurate observation due to the intricacy of the apparatus, clear evidence as to the performance of these organs was not obtained, yet indications that their contents are discharged into the oogonium were not lacking. In any case, however, the presence of the antheridial elements could scarcely be interpreted as necessary for the development of the egg or as in any way promoting its formation. Indeed, the egg apparatus provided with them appeared in general to be considerably slower in maturing and slightly more subject to degeneration than the parthenogenetic apparatus, and it was also more likely to be of extreme dimensions. The antheridial elements are remarkable chiefly because they are exceptionally numerous; rarely fewer than 15 and sometimes as many as 35 were visible on the upper side and about the equatorial plane of the oogonium, and perhaps 10 or 20 more may be concealed beneath the oogonium. The virtual investment of the female organ with such a bristling array of curved structures is one of the striking characteristics of this species.

The oospore is regularly found singly in an oogonium, which it usually occupies almost completely, although there is nearly always a narrow space between the oospore wall and the oogonial wall which occasionally becomes more pronounced in width. The ripe oospore generally has a wall of moderate thickness, and contains a large spherical homogeneous structure somewhat eccentrically placed in a peripheral matrix. Within this matrix granulelike bodies of uniform size are arranged geometrically. They are often 3 or 4 layers deep on one side and 1 or 2 or more rarely 3 layers deep on the other. A strongly refringent homogeneous body oblate ellipsoidal in shape and 5μ or 6μ in diameter by 1.5μ to 2.0μ in thickness, can always be distinguished in the matrix, usually embedded between the outer and inner layer of granular structures, where it appears in profile as an elliptical occlusion. This body, entirely similar to homologous structures in the oospores of Aphanomyces, Pythium, and related genera, may possibly be the nucleus.

Germination of the oospores readily takes place on transfer to water. The oospore wall loses its visibility evidently as a result of

some process of gelatinization (fig. 1, J), after which the contents become distributed throughout the interior of the oogonium. A single stout germ tube corresponding in diameter to the larger vegetative hyphae (fig. 1, H and I), or one or several more richly branching systems of hyphae corresponding to the more delicate mycelial elements (fig. 1, G), may be produced, apparently depending largely on whether the oospore is embedded in a matrix (corn-meal agar, in the trials under discussion) or is immediately in contact with water. In a few instances the single germ tube appeared to terminate abruptly in a small lobulate sporangium, making it seem probable that the production of germ sporangia, frequent in *Aphanomyces euteiches*, occurs here occasionally.

TAXONOMY

In the taxonomy of the Saprolegniaceae major importance has been assigned to the type of development exhibited by the zoospores as well as to the morphology of the zoosporangium. With respect to the former feature, the tomato parasite reveals complete similarity to Achlya and Aphanomyces; with respect to the latter, a partial combination of the characteristics of both these genera. In Achlya the zoospores are fashioned within a massive sporangium, usually of tapering-cylindrical shape, regular in outline, such apical modification for evacuation as may be present being generally very slight. Zoospores may be produced also by the gemmae that develop in many species. These bodies frequently occur in irregular forms, as is evidenced, for example, by Coker's figures of *Achlya flagellata* Coker (*2, pl. 37, fig. 7*) and *Achlya megasperma* Humphrey (*2, pl. 44, fig. 9*). Gemmae of this type and other structures, like some of the more irregular "Conidienstände" depicted by Maurizio (*6, Taf. I, figs. 13, 27*), show a certain degree of resemblance in shape to the simpler, more openly disposed sporangia produced by the fungus under consideration. However, even the most copiously branched gemmae of Achlya or, indeed, of any recognized genus of the Saprolegniaceae, fail to exhibit the measure of complexity and involvement found in the better developed sporangia of the fungus discussed in this paper. These latter, moreover, can scarcely be regarded as "Hemmungsbildungen," to which Klebs' (*5*) researches have reduced many of the structures designated as gemmae. Their production is most luxuriant from fresh mycelium that previous to its transfer to water has been well nourished and is not thereafter exposed to serious bacterial contamination—conditions under which development would not ordinarily be arrested. Nor do they require a resting period for zoospore production like the generality of gemmae. They are essentially short-lived bodies that in the absence of conditions suitable for the fashioning and discharging of zoospores soon degenerate, or, at best, proliferate vegetatively after a promiscuous manner.

With respect to the vegetative thallus a pronounced difference between the tomato parasite and Achlya is evident, the mycelium of the former being, of course, decidedly smaller in diameter. On the other hand, the similarity to the thallus of Aphanomyces is striking, not only as to the thickness of the hyphae, but also as to the general appearance under the microscope. This obvious resemblance, together with the uniseriate production of zoospores in the efferent

hyphae, would seem indicative of a close affinity to that genus—an affinity that finds a peculiarly apt parallel in the genus Pythium, assuming for the latter the wider limits more generally adopted. The genus Pythium includes species entirely analogous to Aphanomyces, in which externally undifferentiated filaments serve as sporangia, as well as a series of forms like the widely distributed parasite, *P. aphanidermatum* (Eds.) Fitz., in which sporangia are represented by communicating systems of distended digitate or lobulate elements, often intricately involved—closely similar in composition to those described in this paper. Schröter (*8*) regarded the Pythium types possessing purely filamentous sporangia as sufficiently distinctive to establish a separate genus, Nematosporangium. As has been pointed out, such a disposition might prove profitable if accompanied by the addition of another genus including the forms with lobulate zoosporangia (*3*).

Assignment of the present fungus to Aphanomyces, to which it seems most closely related, could apparently be accomplished only in contravention of the feature most distinctive of that genus. The adoption of a new genus would seem less objectionable, especially as it is not improbable that as more terrestrial types of Saprolegniaceae come to light other forms of similar morphology may require taxonomic treatment. Such a genus is therefore proposed here under a name meaning "plaited coil"; and it is hoped that a further characterization of the fungus may be conveyed in the specific name suggestive of the abundance of antheridial elements.

DIAGNOSIS

Plectospira, n. g.

Mycelium slender, sparingly or moderately branched. Zoosporangia composed of inflated elements, often compacted into an irregular complex, within which zoospores are differentiated in two or more series, together with a prolonged filamentous element within which zoospores are formed in one series and by which the entire organ is evacuated. Zoospores encysting at the mouth of the efferent hypha, later escaping from their cysts and swarming. Oogonia intercalary or terminal. Antheridia absent or present. Oospores single and somewhat eccentric (subcentric) in internal structure.

Plectospira myriandra, n. sp.

Mycelium 1.8μ to 6μ in diameter. Inflated elements of sporangia 6μ to 18μ in diameter; efferent hyphae usually 5μ to 10μ at base, generally tapering more or less to a diameter 3.5μ to 4.5μ at tip. Sporangia sometimes very extensive and compound; then provided with plural efferent hyphae, each delivering up to an approximate maximum of 500 zoospores. Zoospores, after encystment, 6μ to 12μ in diameter, usually 9μ to 10μ, developing a papilla 2.5μ to 3μ in diameter and 1μ long, the cylindrical wall of which after evacuation persists on the empty cyst wall. Oogonium mostly terminal on short branches, more rarely laterally intercalary or intercalary, subspherical, smooth, 15μ to 33μ in diameter, usually 23 to 29μ, provided with a wall generally approximately 0.5μ, more rarely up to 1μ in thickness. Antheridia absent, or frequently 25 to 55 in number, mostly rudimentary, the smallest approximately 3μ in diameter and 5μ in length, often without delimiting septum; the largest, up to 6.5μ in diameter and 25μ in length, delimited by septum and often potentially functional in appearance; mostly straight, distended cylindrical or curved cylindrical; diclinous in origin, borne in close arrangement on a number of branching systems arising from delicate hyphae. Oospore, single, 13μ to 30μ, usually 20μ to 27μ in diameter, provided with a wall 1.1μ to 1.9μ, usually 1.5μ in thickness, slightly eccentric in internal structure. Mildly parasitic, causing discoloration and death of rootlet tips of *Lycopersicum esculentum* Mill. in greenhouse at Arlington Experiment Farm, Rosslyn, Va., in May, 1926.

In the foregoing diagnosis of the genus Plectospira, as generally in the definition of taxonomic groups within the Saprolegniaceae, more stress is laid upon the morphology and development of the structures

concerned in asexual reproduction than upon the morphology of the sexual apparatus. Except for the internal structure of the oospores, the degree of importance to be attributed to the latter will remain problematical until congeneric forms have been discovered. The presence of antheridial elements in such abundance as to envelop the oogonium to a considerable extent, while not common, is far from being unknown among other genera of the Saprolegniaceae, rather extreme conditions of such envelopment being found, according to Von Minden (7), in *Achlya prolifera* (Nees) deBary and *Achlya oblongata* deBary. Among the forms with single oospores, some species of Aphanomyces show a certain degree of such envelopment, more, however, because of the relatively large size of the several antheridia present than because of their number.

A condition much more similar to that prevailing in *Plectospira myriandra* was described by de Wildeman (9) in an account of a fungus he found parasitic on the oogonia of Characeae and designated as *Achlyopsis entospora*, the type of a new genus. The oogonia were characterized as globose or elliptical, formed terminally on branches, and separated from the thallus by a thick septum often inserted at some distance from the swollen portion. Numerous antheridial filaments derived apparently from several mycelial hyphae sometimes completely invested the oogonium "comme d'un réseau ou d'une couche continue." The abundance of antheridial filaments was held to suggest Achlya, but because of the oospores occurring singly in the oogonia and the development of the latter within the host—two characters which the author regarded as exceptional in the Saprolegniaceae—the fungus was referred to the Peronosporaceae. No sporangial stage was recognized as associated with the parasite. In another portion of the text, however, the author described certain structures also found occurring within the affected oogonia of Characeae:

"Comme le montrent nos figures, ces mycéliums internes etaient constitués par un gros filament ramifié, contourné en tous sens et formant une masse pelotonnée plus ou moins volumineuse. De certaines portions de ce thalle coralloïde naissaient des filaments étroits s'enfonçant dans le milieu; d'autres portions naissaient des ramifications, primitivement assez larges, s'amincissant progressivement, traversant la paroi de l'oogone et se prolongeant dans l'eau."

Although de Wildeman recognized these coralloid structures as closely similar to certain mycelia produced by *Plasmopora viticola* (B. and C.) Berl. and De T., a comparison of his figures (9: Pl. XI, figs. 1–4) with Figure 1, B–E, reveals a much more complete similarity. There can, indeed, be little doubt that the "thalles coralloïdes" represent evacuated zoosporangia of the same external morphology as that characteristic of the tomato parasite. Some consideration, therefore, was given to the plausibility of a taxonomic disposition whereby this parasite was to be assigned to de Wildeman's genus appropriately emended by referring the coralloid structures to it and assuming for them the rôle of zoosporangia with Achlya-like development. It appeared best, however, to reject this alternative, as de Wildeman reported among certain other Phycomycetous forms present in the same material as his *Achlyopsis entospora*, all apparently in rather intimate confusion, two new species of Pythium, *P. characearum* and *P. gibbosum*. These species also were based solely

upon their sexual stages. In the absence of information to the contrary, the probability is presented of the empty "thalles coralloïdes" representing evacuated lobulate zoosporangia of one or the other of these species of Pythium—a probability quite as strong as that they were associated with *Achlyopsis entospora* and represented zoosporangia that had become evacuated after the manner described in this paper for the tomato parasite. That the species to which the binomial *P. characearum* was applied may be a form with lobulate zoosporangia is made more probable by the description of its mycelium as "très abondant, il forme dans le milieu, des pelotes de filaments très enchevêtrés; il semble porter des grappes d'oosporanges."

The structure of the oospore of *Achlyopsis entospora*, with its very thick wall, and its more or less homogeneous contents containing 1 to 4 refringent globules, suggesting in these details little similarity to the structure of the oospore of the fungus affecting tomato rootlets, provides an additional reason for not referring the latter to de Wildeman's genus. As, however, the figures given by that author indicate that his material may have suffered considerable degeneration, it would be inadvisable to attribute too much significance to a comparison of such features.

SUMMARY

Two water molds have been found causing discoloration and death of occasional rootlet tips of tomato seedlings in greenhouses. One was identified as *Aphanomyces euteiches*, known previously as a cause of root rot of peas. The other is described as the type of a new genus resembling Aphanomyces in general habit and in zoospore development but differing from it in having a differentiated zoosporangium composed typically of an involved complex of inflated elements, and a filamentous efferent hypha.

LITERATURE CITED

(1) Brown, W.
1924. two mycological methods. Ann. Bot. [London] 38: 401–404, illus.

(2) Coker, W. C.
1923. the saprolegniaceae with notes on other water molds. 201 p., illus. Chapel Hill, N. C.

(3) Drechsler, C.
1925. the cottony leak of cucumbers caused by pythium aphanidermatum. Jour. Agr. Research 30: 1035–1042, illus.

(4) Jones, F. R., and Drechsler, C.
1925. root rot of peas in the united states caused by aphanomyces euteiches (n. sp.). Jour. Agr. Research 30: 293–325, illus.

(5) Klebs, G.
1899. zur physiologie der fortpflanzung einiger pilze. II. saprolegnia mixta de bary. Jahrb. Wiss. Bot. 33: 513–593, illus.

(6) Maurizio, A.
1896. die sporangiumanlage der gattung saprolegnia. Jahrb. Wiss. Bot. 29: 75–131, illus.

(7) Minden, M. von
1912. pilze. (Kryptogamenflora der Mark Brandenburg, Bd. 5, Heft 4, illus. Leipzig.)

(8) Schröter, J.
1897. saprolegniineae. (Engler, A., and Prantl, K., Die natürlichen Pflanzenfamilien, Teil 1, Abt. 1, p. 93–105, illus. Leipzig.)

(9) Wildman, É. de
1896. Notes mycologiques. Ann. Soc. Belge Micros. 20: 105–136, illus.

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